

nature*July 10, 1975*

Good for people, bad for science

ONE of the merits of democracy is not simply that it gives people the chance to choose whom they want to govern but also that it gives them the chance to say whom they do not want. In particular the democratic process makes it possible to say to a government—enough, we don't like the way you do things, it's time to try another way. The ability to reject as well as to accept seems to be a necessary part of most human endeavour, at least any endeavour in which there is any concept of growth or evolution. Foolish would be the person prepared to take on any commitment from which there seemed to be no means of escape, if needed, and foolish are those who try to make the means of escape humiliating, expensive or distasteful.

The development of science also shows the rejection mechanism in action; not simply in the process by which the scientific manuscript has to stand the test of peer review, but also in the endless self-criticism which the good scientist applies to his ideas long before they are committed to paper and in the rapid way in which the scientific community works over published material seeking out errors and inconsistencies.

It is strange that, although we recognise the need that scientific ideas should not only have a platform but should also have to face a barrage of rotten eggs, we are unbelievably cautious when it comes to the scientist himself. If the idea doesn't make the grade, it won't last six months; if the scientist doesn't make the grade—well he's got tenure and could be around for another thirty years.

Job security is, of course, an excellent thing in many ways. It reassures those who set out on long and sometimes tedious intellectual pathways that they are not going to have to worry about keeping in favour with their employer or about producing quick results simply in order to stay in business. It keeps the universities and the Civil Service away from any form of external threat to their pursuit of knowledge. But at what cost?

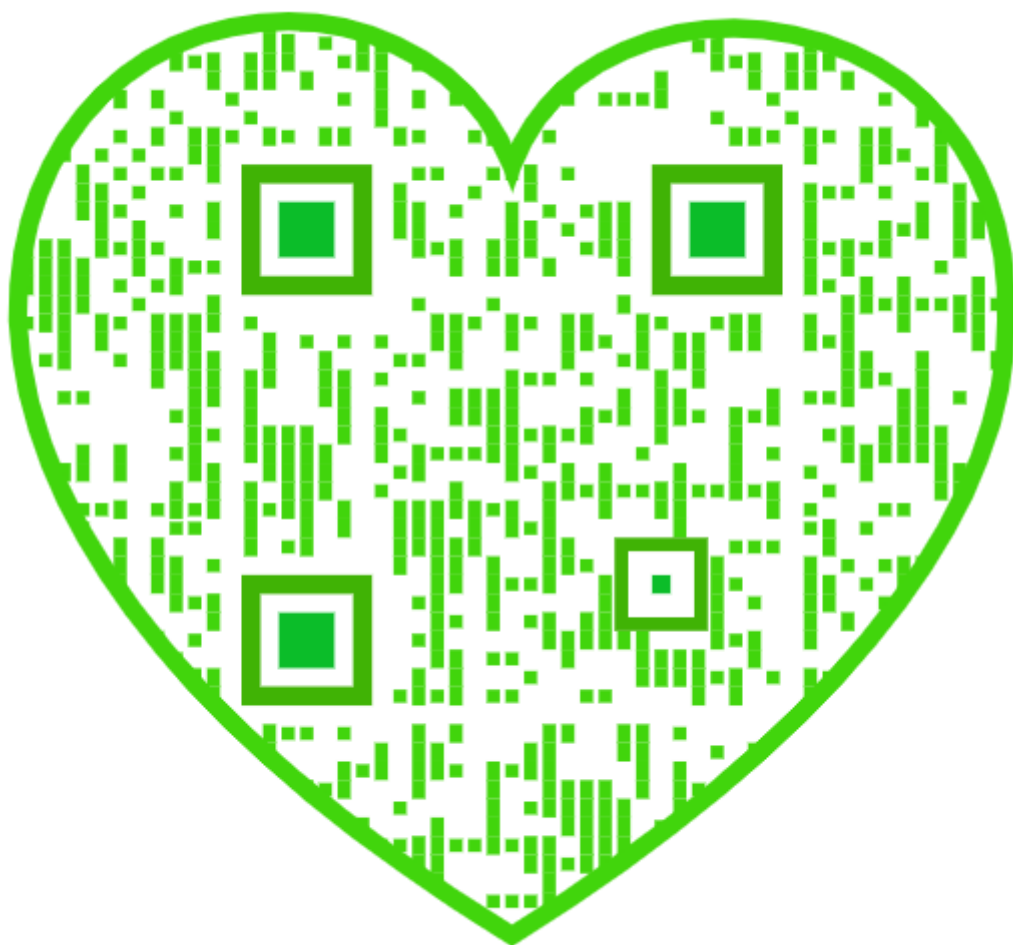
Tenure is a virtual guarantee that the system will either agree indefinitely to your doing what you want to do or that it will find you another job if the present one gets too much. This is a very humane and

enlightened practice but it bears little relationship to a world in which the demands on science are changing rapidly; it also takes no notice of the propensity of science to proceed by revolution. Further, although keeping in favour with one's employer is not necessary to being a good scientist, science, even within universities, does call for a certain amount of management, and the need for management is bound to increase. Yet if managers lack the ultimate sanction of being able to dispense with those who cannot or will not contribute effectively, management has one hand tied behind its back.

The pressures in recent years have been rather obviously towards increasing job security in universities (the Civil Service is already about as secure as the priesthood) and there is talk now of giving laboratory technicians some sort of tenure. Such pressures ought to be firmly resisted and, if anything, there should be serious discussions about reversing the trend. It would obviously be silly to put the academic or governmental scientist on to a three-months' notice basis, but few could complain of indecent haste if a contract came up for renewal on both sides once every ten years. There might be stronger support for such an idea within the scientific community than is generally realised.

Such a procedure would also permit the at present almost impossible operation of closing down a department to be carried out relatively bloodlessly within the space of a few years when necessary.

The standard objection to any loosening of the tenure grip is to point to the problems of those who would be turned out after many years of service. The answer to that is that there are already many careers in which there comes a day of reckoning; perhaps the most analogous is the military profession. If the change is intelligently anticipated and financially assisted there is no reason why it should be a humiliating experience at all. Indeed, for all we know, the release every year of a few hundred scientists into a more general environment might encourage industry and commerce to use them imaginatively. After all, not every retired Major runs a sweet shop. □



IFIAS: the top people's problem posers

The International Federation of Institutes for Advanced Studies aims to mobilise a global intellectual community to examine problems which have not been officially recognised and subjected to political convenience. Wendy Barnaby reports.

THE last few years have seen an upsurge in discussions about the social responsibilities of scientists. From Koestler's "Callgirls" to articles in the daily press, the familiar message has been that science is not an activity which should be broken up into discrete categories and carried on in laboratories isolated from moral issues and awareness of the directions in which societies are developing. Such sentiments have already—unfortunately—become platitudinous. So it is rather a shock to discover a potentially high-powered organisation which exists to put them into action.

The International Federation of Institutes for Advanced Study (IFIAS) is a loosely-structured conglomeration of some twenty research institutes in sixteen countries. It is a unique federation because the institutes cover at least ten very different disciplines and co-operate on scientific projects which they hope will contribute to the solution of some of our global problems.

The federation was set up in 1972 with \$90,000 in equal grants from the Nobel, Rockefeller and Swedish State Bank Foundations. It is non-profit making, and non-governmental, with headquarters at Stockholm in three small rooms in the Nobel House, where its secretary, Dr Sam Nilsson (a physicist), also lives. Its structure and methods of working are very flexible: it is not so much an organisation, says Dr Nilsson, as a lack of organisation. General guidance on the course to be followed and on specific projects is given by the directors of the member institutes, a board of trustees (whose Chairmen are Drs Alexander King (UK), Paul M. Fye (USA), Abdus Salam (Pakistan) and Victor Urquidí (Mexico)), and a panel of advisers in different fields who have participated in IFIAS work. The secretariat is the go-between for the institutes, and initiates and catalyses new projects. It has been supported, after the exhaustion of the initial funds, by

\$150,000 a year, a sum made up of annual fees from member institutes, each of which pays 0.5% per 1,000 units (whether dollars, yen, rupees or whatever) of its operating budget, and grants to be paid for 5 years by foundations and similar bodies in Sweden (\$35,000 each year), USA (also \$35,000), Japan (\$10,000), Italy (\$5,000) and France (\$5,000). Each institute taking part in a project also pays a levy of 10% of its contribution to the project. These levies are the secretariat's third source of income.

The institutes themselves specialise in fields including oceanography, medicine, physics, humanistic studies, economics, ekistics (the study of human settlements), genetics, international relations, rice research, mathematics and insect physiology and ecology. Together they represent 5,000 researchers and over \$100 million in annual expenditures.

IFIAS' intention is to mobilise this global intellectual community to examine problems which have not been officially recognised and subjected to political convenience. It is the belief of the federation that more effective communication must be stimulated between, on the one hand, those who make the decisions involving long-term consequences for mankind, and, on the other, the world's intellectual and spiritual leaders. By choosing as yet politically uncontaminated areas of study, and by consulting and involving the decision makers in projects from the very beginning, IFIAS hopes to bring the influence of facts and well-considered alternatives to bear on the decision makers when they have to act. The emphasis is on working with decision makers rather than presenting them with tomes of conclusions which will never be read. By making use of the contacts of its members, the federation has been able to adopt a policy of dealing only with top people, whether politicians, bureaucrats, businessmen or scientists, and so has a greater potential for influence than most organisations.

Specific action requires a specific focus. Every project is therefore concrete, complex, budget- and time-limited, global in scope and involving inter-disciplinary research. Participation in projects is not limited to member institutes. Other research bodies take part when their expertise is needed, and in this way various UN agencies—UNESCO, WHO, the UN Environment Programme (UNEP) and the World Meteorological Association (WMO)—are involved in different projects. No proposal for a new project is accepted before it has been extensively screened at workshops to make sure that nobody else is doing it better, that at least two member institutes from different

countries are willing to assume responsibility for it, that a specific scholar is willing to lead it, and that it has social, ethical and humanistic implications and financial support. When a topic is first accepted, IFIAS sets up a programme of activity which may or may not develop into a full-scale project. So far, funding has come from the World Bank, Volkswagen, the Ford and Rockefeller Foundations, UNEP, UNESCO and the Swedish agency for aid to developing countries (SIDA). To promote contacts with those who wield power over hundreds of thousands of lives, and to attract funds, IFIAS has also begun to approach international corporations.

The programmes and projects so far in operation are:

- Impact of climate changes on the character and quality of human life (budget: \$300,000)
- Human settlements: understanding their nature and guiding their development for the benefit of man (\$325,000)
- Socio-economic and ethical implications of enzyme engineering (\$194,000)
- Energy and quality of life (\$80,000). These programmes and projects are being screened:
- Options for regions faced with water shortage
- Interaction of health, nutrition and education on human growth and development
- Loss of productive soil
- Third world: cultural transfer—cultural change
- Independence and interdependence in the face of regional collapses.

The projects are not concerned with research which gives rise to predictions about resources, climate changes or urbanisation for example. As Dr Nilsson points out, there is a wealth of such predictions already. IFIAS sees its task as examining their consequences. The human settlement project starts from the prediction that by the year 2000, 50% of the estimated world population of 6.5 billion will live in cities. The rationale of the project is that, as these cities will be built anyway, they should be planned in order to conserve resources and promote the best possible living conditions for their inhabitants.

The project aims to draw up a classification of human settlements and, through case studies from Sweden, France, Mexico, India, USA, Columbia and Argentina, to establish criteria for understanding their development. It is hoped that the project will arrive at a conceptual framework which could point to practical suggestions for the UN Conference-Exposition on human settlements (HABITAT) to be held in Vancouver next year.

The project on climatic change is

being carried out against the background of predictions that climatic alterations will soon threaten existing systems of food production. In the face of conflicting predictions about the changes and their effects, the project has adopted the general IFIAS method of dealing with a range of predictions varying from the least to most drastic. What is 'drastic' to begin with is then defined by the general criteria for project selection, mentioned above. The researchers are aiming for plans and actions to establish the technical, social and political means to cope with different climatic threats to food.

The energy programme is in two parts. The first is concerned with energy analysis, which is the idea of working out how much energy is embodied in goods or services, expressed as physical units. Undertaken in the light of predictions that, because of limited resources and extravagant use, energy may become a severely limiting factor in many sectors of society, energy analysis includes not only the direct energy used for fuel and to run machinery, but also the energy required to make available each of the input materials. For example, in an industrialised country a loaf of bread is the end product of seed which grows through the absorption of energy (partly from natural sources, partly from petroleum made into fertiliser) into grain, which is harvested, transported and processed into flour by machines, then transported again and added to other ingredients (all of which have arrived at this point through similar processes), mixed and baked by more machines into bread (which is possibly wrapped in paper that is the end product of another energy-consuming chain), and finally transported by petroleum-eating lorries to the supermarkets.

Because energy analysis is value-free, it can provide information, in addition to the predictions of our all-too-fallible economic theories, for decision making. It appears to be neither costly nor difficult to carry out, as much of the data needed already exist in national statistics, industrial process data and various other sources. The urgent need is for a common set of rules for calculating energy inputs and transformations, and for common terminology, symbols and units of measure. It is these that the IFIAS project is drawing up.

Once the tools are agreed upon, researchers in different countries will be able to calculate the energy inputs into similar products and identify steps where the use of energy could be reduced. Or the analysis could be used to compare the anticipated energy saving of a development—house insula-

IFIAS Member Institutes

Aspen Institute for Humanistic Studies, New York.
Athens Center of Ekistics.
Center for Education in International Management, Geneva.
Center for Theoretical Studies, Miami.
El Colegio de Mexico.
Department of Cell Research and Genetics, Karolinska Institute, Stockholm.
The Graduate Institute of International Studies, Geneva.
Instituto de Biofísica, Rio de Janeiro.
International Centre for Theoretical Physics, Trieste.
The International Centre of Insect Physiology and Ecology, Nairobi.
The International Rice Research Institute, Manila.

The Japan Economic Research Center, Tokyo.
Johnson Research Foundation, Philadelphia.
Mathematics Institute, Coventry.
National Institute for Research Advancement, Tokyo.
Niels Bohr Institute of Physics, Copenhagen.
Institut Pasteur, Paris.
Tata Institute of Fundamental Research, Bombay.
University Corporation for Atmospheric Research, Boulder, Colorado.
The Walter and Eliza Hall Institute of Medical Research, Melbourne.
The Weizmann Institute of Science, Israel.
Woods Hole Oceanographic Institution, Massachusetts.

tion, for example—with the initial energy investment necessary to create the development. The energy impact of recycled materials could be assessed. And industries and governments would be able to examine the energy implications of their decisions.

The second part of the energy project is entitled "Alternative Choices of Level of Energy Consumption in Different Societies". Although it is usually taken for granted that a linear relationship exists between energy consumption *per capita* and GNP *per capita*, this is actually true only in energy-affluent societies. The project is analysing why a certain country can achieve the same GNP *per capita* as another country but with a much lower consumption of energy. The study is aiming at comparative analyses of different socio-economic trade-offs in different countries (for example Scandinavia, Japan, India, Kenya) at varying levels of energy consumption rates (say 4%, 2% and 0% a year).

The project on enzyme engineering is typical of the scope and, in its relevance to developing countries, of the emphasis of IFIAS work. Enzymes are of course already widely used on a 'use-and-throw-away' basis to support a variety of socially necessary processes. But it was not until techniques were developed for immobilising enzymes on a support material that their catalytic activity could be retained for reuse, and this has reawakened interest in the use of enzymes as catalysts in many different areas. The IFIAS project is led by Professor Carl-Göran Hedén of Stockholm's Karolinska Institute and is being carried out by member institutes in France, Israel, India and the USA, as well as other institutes in the USSR, Sweden, Canada and the UK. UNESCO is also participating. The project is investigating the use of enzyme engineering in four fields: developing

enzyme-catalysed energy transfer devices which could increase the practical applications of fuel cells or use electrical energy to synthesise organic chemicals for industry, nutrition and pharmaceuticals; testing a quick, enzyme-dependent method of detecting tropical diseases such as malaria; developing a technique of giving the booster shot at the same time as an initial injection in immunisation (to solve the problems of finding people in developing countries when their booster is due); and improving biological control agents by means of enzyme attachment to insect pathogens.

Professor Hedén is enthusiastic about the potential of enzyme engineering in other areas as well. At a symposium in Tokyo last September he outlined an idea which entails a better use of resources for accelerated development in the Middle East. Pointing out that the methods of chemical catalysis currently used in the production of methanol need such high pressures and temperatures that only large-scale factories are competitive, he proposed the use of enzyme catalysis in conjunction with natural methane flares, which today burn as waste, to produce methanol in small-scale plants. Such methanol could be used not only for making fodder protein and enzymes, but also as a cheap, high-octane and relatively clean fuel for cars, or as a safer medium than methane for the carriage of hydrogen.

Such "equilibrium technology"—processes and industrial equipment which use renewable energy sources and are geared to a maximum of recycling of matter—is typical of the inventiveness and optimism, some would say idealism, of the IFIAS approach. The attractiveness of Professor Hedén's scheme for the Middle East, for example, does not end with methanol technology. It also emphasises a range

of biological uses for solar energy ranging from waste treatment to fish farming. He envisages Arab countries such as Kuwait, whose national development plan is very conscious of, and aims for, a balance between ecosystems, providing the vision and financial resources for a regional development effort based on an international input of scientific manpower. This would help to make each side in the Middle East conflict dependent on the other for its future.

As none of IFIAS' projects has yet been completed it is too early to talk about their final results. Already, however, there are signs that IFIAS' work is not going unnoticed. The Brussels headquarters of the Common Market has asked IFIAS for information about the energy analysis programme, and has reserved \$100,000 for a joint IFIAS/Common Market project on energy. The Market is particularly interested in adopting IFIAS' methodology for working out alternative levels of energy consumption, with a view to using the technique in drawing up a European energy policy. The World Health

Organisation is also very interested in an IFIAS project; this time the one on enzyme engineering. WHO's attention is concentrated on that part of the project dealing with the new method IFIAS has developed for diagnosing tropical diseases. The method was tested in East Africa last February and looks promising.

The first evaluation of IFIAS will no doubt be the one planned by the organisation itself, to be conducted in 1977—five years after its inception. This idea is good in principle, but should not be expected to produce any rational calculation of its achievements. One of the problems involved is the lack of any concrete output or of any yardstick to measure it. Barring administrative incompetence, each team of institutes will obviously be able to produce lengthy documents supporting the results of its particular project. By then they will also perhaps be able to point to even more examples of their work being referred to by the decision makers.

The continuation of IFIAS will depend not on the results of 'evalua-

tions' of its work but on the enthusiasm of its members. And nothing could be calculated to sustain that enthusiasm more than the sort of successes the energy analysis and enzyme engineering projects have already enjoyed. Convincing decision makers of the need for action may be harder in the project on climate change. The invisibility of the problem makes it an unlikely target for that public concern which, in countries where politicians depend on the people for their positions, can be an effective complement to IFIAS' high-level approaches in making the decision makers act. On the other hand, the problems dealt with in the project on human settlements are symbolised by the ugliness, dirtiness and congestion of modern cities. If handled properly, the project could have an impact on public policy. Unfortunately, the impact will be greatest if IFIAS keeps its activities separate from the UN conference towards which they are aimed; for incisive and intelligent proposals have a way of emerging from the politically-sensitive and grindingly slow UN machine in a very emaciated form. □

SOMEHOW or other, they had cajoled the money and effort to try their strange and fabulously expensive experiment. Many people said that the funds should be spent on something more useful, or not spent at all. But there was a great public curiosity in the idea; perhaps Orson Welles had helped as much as anyone with his radio broadcast of invaders from Mars in 1938 that caused a nationwide panic. Is there life on Mars? We were now ready to settle for a few anaerobic bacteria rather than green monsters with fusiform antennae.

The Viking biology team had been called together by the team leader, Harold Klein, for the last time before the launch on August 11, 1975.

One year ago, our hope that even one of the three experiments would be ready by launch-time was a slender one. The constraints were severe; everything that is sent to Mars must be sterile. There is no use carrying bacterial spores from Earth to Mars to detect their presence later. It cost about a million dollars to sterilise the parachute, principally because no-one had ever sterilised a parachute. "Hardy bugs" kept showing up, including one whose spores lived for 6 days at 125 °C. The launch date had to be just right for slinging a shot through the heavens to intercept a planet in conjunction. Worst of all, the instruments were unprecedented in design, infinite in complexity, and strictly limited in size and weight. In engineers' terms, they cost a million dollars per pound; a compacted 20-kilogram mass of electronic and

chemical gadgetry whose price made crown jewels look like dime-store baubles. One of the experiments is called "labeled release". It will incubate a pinch of Martian soil, moistened with water containing radioactively tagged nutrients (formate, glycine, DL alanine, DL lactate and glycolic acid)

Viking gets ready

from Thomas H. Jukes

for two weeks at 6°–14 °C, and any emitted gas will be monitored for metabolically-produced radioactivity.

The second is "pyrolytic release", based on the idea that photosynthetic fixation of carbon dioxide, and possibly carbon monoxide, should take place biologically under Martian conditions. The surface sample will be exposed to "artificial sunlight" from a xenon lamp for several days in an atmosphere containing ¹⁴CO₂ and a little ¹⁴CO. The soil will then be heated to 600 °C to destroy organic compounds resulting from photosynthesis and the emitted gases will be measured for radioactivity. The experiment is designed also to detect dark fixation of ¹⁴CO₂ and ¹⁴CO. This experiment is specifically oriented towards Martian organisms that may differ from terrestrial ones in being intolerant of water or "terrestrial nutrients". The third experiment is named "gas exchange" but is often called "the chicken soup experiment" because it

frankly seeks to woo Martian bugs with a rich, non-radioactive meal containing amino acids, vitamins and co-factors, salts and other nutrients, from kitchens on the Earth. The soil sample is moistened with the culture medium, and the atmosphere in the chamber, mostly helium, is periodically analysed by gas chromatography to see if gases such as methane and CO₂ are emitted.

At the meeting on June 17, the engineers from Thompson-Ramo-Woolridge and Martin-Marietta appropriately handed us a glossary of 80 acronyms to help us follow their talks. The mood was one of relief, and guarded optimism. All three of the experimental instruments, miraculously, had come into working order at the last minute. The Viking Biology package, in duplicate, had been loaded into two spacecraft at the Kennedy Space Center in Florida. All that now remains is that the instruments should survive the launches on August 11 and 21, the eleven-month, 450-million-mile journey, and the "soft" landings through the thin (5 millibars) CO₂ atmosphere of Mars in July 1976.

After that, perhaps, the faintly-whispered signals will tell us what is happening; not only in the biology package but in the colour cameras, the water detector, the infra-red thermal mapper, the gas-chromatograph-mass-spectrometer, the X-ray fluorescence spectrometer, the seismometer and the weather station. But a main worry is: if the signals next year say that life may be present, will people believe the message?

international news

REPORTS, analyses, blueprints and predictions on energy policy have been pouring out of government agencies in the United States at such a rate during the past years or so that they now often raise little more than a flicker of public interest before being confined to the bookshelves to gather dust. But a report published last week by the Energy Research and Development Administration (ERDA) has attracted considerable attention, and rightly so.

The first broad statement on energy policy to emerge from ERDA since the agency was established in January, the report reflects an important change of priority among long range energy programmes. Specifically, ERDA recommends that more emphasis should be placed on solar energy and thermonuclear fusion, and less on the liquid metal fast breeder reactor (LMFBR), for meeting energy needs towards the end of the twentieth century and thereafter.

Moreover, ERDA has recommended that funding for a variety of non-nuclear programmes, such as the production of synthetic petroleum and natural gas from coal and the development of technology for exploiting geothermal energy, should be increased immediately, while the nuclear programme should be revamped to give more support to research and development on such items as reactor safety, safeguards and the disposal of radioactive wastes.

Described by Dr Robert C. Seamans Jr, the Administrator of ERDA as "laying the groundwork" for energy research and development planning, the report also tacitly abandons the Project Independence goal of reducing oil imports to zero by 1985 (a goal which has long been considered unrealistic in any case). Instead, the report suggests that the United States is unlikely to become self-sufficient in energy before about 1995, unless there is a significant, and unexpected, change in the American way of life.

The report begins by noting that the United States now relies most on the least plentiful domestic energy resources to meet its burgeoning energy requirements. Petroleum and natural gas account for nearly 75% of total energy consumption, yet the Department of the Interior and a committee of the National Academy of Sciences recently suggested that domestic oil and gas supplies are likely to dry up in

US puts energy plans in several baskets

by Colin Norman, Washington



Seamans: laying the groundwork.

about 35 years. Consequently, a switch must be made to other energy sources as swiftly as possible, but Seamans pointed out last week that previous transitions to new energy sources—from wood to coal in the nineteenth century and from coal to oil and gas since 1900—have taken about 60 years to complete. It is clear, he said, that "we don't have 60 years to transfer another energy source" this time.

First, however, a word about what the report is not. "What you will find here is not an instant remedy for energy problems", Seamans said, "nor can it be looked at as an exact blueprint" because priorities will inevitably change as research and development uncovers problems and opportunities in exploiting new technologies. Rather, it is an assessment of present priorities in energy research and development, and ERDA will update the report each year.

The report also makes no explicit projection of energy demand; instead, it outlines the steps which seem neces-

sary to meet whatever demand arises. That approach, it should be pointed out, ignores such considerations as whether it would be better to reduce energy demand rather than open up new resources—a consideration which many see as the crucial question in energy policy for the United States. The report simply states that energy policy should "provide for future needs so that future life styles remain a matter of choice and are not limited by the unavailability of energy".

The conventional wisdom in government energy planning has essentially been that increased use of coal will help bridge the gap between supply and demand of energy in the short term, and that nuclear power will gradually take over from oil and gas for electricity generation in the longer term. The ERDA report certainly does not abandon that view of things, but it suggests that a much greater role should be played by such exotic energy resources as geothermal energy and solar power. It offers a set of recommendations to help increase domestic energy supplies in the near term (between now and 1985), the mid-term (1985 to 2000) and the long term (early in the twenty-first century).

● For the near term, the report suggests that highest priority should be given to efforts to expand direct use of coal, to increase the proportion of electricity generated by light water reactors, and to enhance the recovery of oil and gas from new and existing deposits. Equally high priority should be given to energy conservation by increasing the efficiency of transportation, improving the insulation of buildings and extracting energy from waste materials.

Some changes of emphasis are needed in existing research and development programmes to help achieve those goals, ERDA suggests. Most important, the report recommends that some money should be taken out of the budget for the LMFBR programme and used instead to help improve the safety and reliability of light water reactors, and to develop safe methods for disposing of radioactive wastes. Research and development aimed at mitigating the environmental impact of coal burning should also receive more attention, the report suggests.

● For the mid-term, ERDA suggests that highest priority should be given

to the development of technologies for producing synthetic petroleum and natural gas from coal, and extracting oil from shale. President Ford announced in January that the Administration has set a target of producing 1 million barrels of synthetic petroleum by 1985, a goal which ERDA says can be achieved only if joint government-industry arrangements can be worked out to ensure that new technologies are swiftly taken up for commercial production. The report also recommends that research and development on *in situ* methods of extracting gas from coal and oil from shale should be stepped up because such methods would cause less environmental destruction and require less water. Equally high priority should be given to increasing the use of geothermal energy for electricity production, and the use of solar energy for heating and cooling buildings. Those resources "promise a significant impact" for the mid-term and beyond, ERDA suggests, but they are at present under-used and under-funded.

● It is in the assessment of priorities for the long term, however, that the report recommends the most significant policy changes. ERDA suggests that the LMFBR programme, the nuclear fusion programme and the develop-

ment of technologies for generating electricity from solar energy should be accorded equal priority.

For several years, the LMFBR programme has enjoyed pride of place as the single most expensive energy research and development effort supported by the federal government. The goal was to have a demonstration reactor in operation by 1980, to introduce the reactor commercially in the late 1980s, and to have about 400 individual plants in operation by the year 2000. But the programme has recently run into considerable trouble. The original estimate that the research and development effort would cost \$3.3 thousand million has now risen to \$10.7 thousand million, the timetable has slipped, and the programme has met with public concern over the adequacy of safeguards to guard against the theft of plutonium, and fears about the toxicity of plutonium. Because of such problems, ERDA believes that it would be foolish for the United States to place all its eggs in the breeder basket, and it is recommending that solar technologies and fusion be accorded equally high priority.

But that does not mean that ERDA has abandoned the breeder project. Last week, in a separate statement, Seamans announced that ERDA will

publish an updated environmental impact statement on the LMFBR programme in about three months' time. The objective, Seamans said, will be to press ahead as rapidly as possible with the research and development effort. Once that phase is completed, the government will be in a better position to assess the environmental and safety aspects of the LMFBR before giving the green light to commercial production of electricity from the reactor, Seamans said. "There is no presently available or prudent alternative to this course of action," he believes.

Nevertheless, because of a number of factors, ERDA has decided that construction of the first LMFBR demonstration plant, to be built near Oak Ridge in Tennessee, will not begin until late next year, which will put it another year behind schedule. Consequently, Seamans has suggested that Congress should reduce the LMFBR budget for this year by \$60 million and transfer some of those funds to other parts of the nuclear programme.

In order to carry out the recommendations outlined in its report, ERDA has suggested that Congress should increase its budget for various non-nuclear programmes by \$143 million for this fiscal year (which began on July 1). The budgets for fusion and solar energy should be increased by \$38 million and \$19 million respectively. ERDA suggests, while research and development on fossil fuels, geothermal energy, conservation and such advanced energy systems as magnetohydrodynamics should also receive substantial increases.

The requests will certainly not fall on deaf ears. In fact, Congress will probably be even more generous towards some programmes than ERDA wants it to be. Last month, the House of Representatives passed a budget bill for ERDA which would add \$158 million to ERDA's budget request for non-nuclear energy programmes, including an additional \$43 million for solar energy. The bill has yet to be passed by the Senate, but similar action is anticipated there.

Finally, Seamans noted during his press conference that often "people say, 'well, we ought to be able to solve this problem. After all, we got a man to the Moon in 10 years'", but he pointed out that "It is one thing to carry out the development and take a relatively small number of astronauts on highly experimental type missions. It is another thing to put into being in this country an energy delivery system, an energy conservation system that will satisfy the needs of over 200 million people". Seamans was Deputy Director of NASA during much of the Apollo programme. His new job will clearly be more challenging. □

SUPPORT of basic research in the United States will decline by about 8% in 1975, largely because of inflation, according to a report published by the National Science Foundation (NSF). The foundation calculates that government, industry and non-profit institutions will provide between them about \$4,100 million for basic research this year, an increase of about 2% over last year's expenditures but an insufficient amount to keep pace with an inflation rate of 10-11%.

Overall, however, the picture does not look too depressing, and it is certainly much better than in many other countries where inflation rates are nearer 20-30%. The NSF calculates that total spending on basic research, applied research and development will climb to about \$34.3 thousand million in 1975, which is an increase of 7% (or a decline of 3% in purchasing power). The development end of the research and development spectrum will fare best, reflecting the increasing support of energy programmes and the rapid increase in outlay on the space shuttle. Moreover, for the first time, industry is expected to spend as much on development as the federal government—about \$11 thousand million each.

The decline in real support for basic research will reduce the purchasing power of the research budget to about the same as it was in 1969, and it follows several years of essentially no growth. Although the NSF does not break down the budget between fields, it suggests that outlays on military and space research and development will account for a slightly smaller share of the total—about 36%, compared to about 50% in the mid-1960s. Outlays in those two areas are expected to be a mere \$12.3 thousand million in 1975.

The analysis is based partly on the Administration's budget request, which was submitted to Congress in February. Although Congress has yet to complete action on any major appropriations bill (even though the fiscal year has already started) the indications are that it will increase the total federal outlays for applied research and development while reducing expenditure on basic research even further. Last month, for example, the House of Representatives deleted some \$44 million from the NSF's budget, but increased the applied research budgets of the Energy Research and Development Administration and the National Institutes of Health. The Senate is expected to follow suit.

THE inaugural meeting of the World Food Council was in danger of breaking down in Rome last week after delegates of the developing countries had expressed dissatisfaction with the draft final report prepared by the secretariat. A major row blew up as the secretariat was accused of uncooperativeness and inefficiency, among suggestions from delegates that the Council was dominated by 'imperialistic and colonial forces.'

"Possibly the best thing that could have happened" is how a senior FAO staff member described the row that brought the WFC meeting into the headlines. Just what sparked it off is not clear. One factor was certainly the frustration of delegates—among whom were ministers from some 30 countries—at the inadequate preparation for the meeting, and the inefficiency and tactlessness with which it was handled. Another was probably the feeling among the Third World countries that the United States was trying to bulldoze the meeting into accepting its view of how to deal with world food. In any event, a meeting which, after a week's argument, finds it necessary to appoint a working party to decide its own rules of procedure, and accepts a declaration charging its president with convening a further session "within a reasonable period" cannot be said to have helped put a single spoonful of rice into the mouth of any one of the world's hungry millions.

In the eyes of many people, there is little need for another new secretariat to operate a World Food Council, but if it must be set up, it should not draw something like half its top staff from the United States—an unnecessarily tactless way of reminding the hungry countries who they depend on for extra food. One thing that has to be clearly seen is that responsibility for the recent farce in Rome rests not with the impatient delegates of the Third World, nor with their exasperated opposite numbers from the rich countries, nor with the FAO, but squarely on the shoulders of the UN in New York. Coming as it does under UN, the new World Food Council is seen as a political body, and one that, as another senior FAO man put it, "has nothing to do with the old, endemic, stratified, perennial and above all, undramatic, world food problem. You can't go on dealing with a long term problem only when events (such as the Sahel disaster) force the politicians to notice it."

Another important factor in this messy situation is the open and long standing disagreement between the FAO, the agency responsible for world food, and UN headquarters in New

York, which is concerned with the politics and economics of the food problem, but hardly at all, it sometimes seems, with the fate of the hungry millions. At the World Food Conference, also convened by the UN and held in Rome last November, it almost seemed that the FAO was about to be dismembered, with some at least of its functions being taken over by the UN—presumably on the grounds that after 30 years of existence, the agency has still failed to prevent people from being underfed, something it was not set up to do in the first place. It was in Rome that Dr Kissinger, in one of the less fortunate pronouncements of his career, stated that the world food prob-

tries which largely control the conditions of international trade, with the exception of oil, provide other constraints. International agricultural adjustment and a more rational world division of labour are needed to increase employment in developing countries as well as their export earnings," said Dr Boerma.

It was worth noting, he added, not only that the international development assistance had conspicuously failed to meet the targets of the Second Development Decade, but also that less than 10% of official development assistance was allocated to agriculture. Calling for food aid to be "deliberately planned as an integral part of world food policy", he reminded the developing countries that "food aid is a bridging measure that will tide them over until their efforts to increase their agricultural growth rate begin to pay off." And finally, the key was to find and implement the means of increasing the purchasing power of the world's peasantry, for the biggest constraint of all is poverty.

Boerma pointed out that, in spite of prospects for a good crop for 1975-76, world food stock would remain dangerously low, and the "most seriously affected" countries would still face a food gap of between 15 and 20 million tons for 1975-76. What is needed is a vast cooperative effort to bring about "general economic and social development on a massive scale that will enable the legions of the poor ... to earn a decent livelihood so that they can buy the food they need."

Dr Boerma remains unconvinced that the World Food Council, at least as at present planned, can bring about these aims in the short (10 year) term. But while he was speaking in Geneva, the first meeting to try to set up an Agricultural Development Fund was going on in Rome. This may prove to be the one solid achievement of last year's World Food Conference. The debate lies between the Arab, oil-producing countries, who have shown themselves willing to put up their share of the cash, the United States, and the EEC. It is the last of these, notably West Germany, who have been dragging their feet, with Britain in the perhaps unusual role of trying to persuade her partners to spend more. At the recent Rome meeting, it was Britain that set the pace with a pledge of a further \$15 million above her already large contribution to the international fertiliser supply scheme. This is an essential part of the effort to help the needy countries produce more of their own food, which, as everyone agrees, is the only way the world food problem can be solved in the long term.

Facing the facts about food

from Peter Collins, Geneva

lem could be solved in 10 years. What is even more unfortunate is that this was accepted by the UN as a basis for the policy of the proposed World Food Council, to the dismay not only of the FAO but also of the many people inside and outside the UN system who are aware that neither the past performance, nor the current statistics, nor the future promise of the world community justified such a statement.

Here at least the record was set straight by Dr Adeke Boerma, the FAO's Director-General, in his unscheduled speech to the recent Rome meeting. He stated bluntly that the professed aims of the World Food Conference "cannot be brought about within the next 10 years." Nor would he like to say when even the target of 3.6% a year for increased food production could be met. Last week, at the Geneva meeting of the UN's Economic and Social Council, Dr Boerma returned to the attack. Pointing out that "bad weather conditions can hardly be blamed" for the long term lag in agricultural production, he analysed the major constraints both within the developing countries, and in the world community as a whole, which have perpetuated the dismal performance of world agriculture for the past 25 years. Many of these are longstanding, highly intractable problems—lack of fertile land and water resources, absence of suitable technology for the many millions of largely illiterate peasant farmers, land tenure systems which militate against increased production by removing all personal incentives, the whole inadequate rural infrastructure in the developing countries. But much of the blame lies outside these countries. "The attitudes in developed coun-

correspondence

Crowther-Hunt's proposals

SIR,—Your leading article (May 29) outlines proposals which Lord Crowther-Hunt anticipates the academic community will eventually endorse. He wants the opinion of university teachers in order to support his own case, but any alteration in the role of universities in Britain will of necessity require discussion among teachers, employers and students.

Lord Crowther-Hunt should ask his colleagues in the government, 'did they suffer or did they benefit from a university education?'

It is facile to quote a figure of 400,000 in full-time tertiary education and the commitment to increase this number to 640,000 by 1981. Has anyone gauged opinions among students, because it would seem that from an economic standpoint there is a distinct disadvantage in pursuing tertiary education. As one graduating student said to me, "I feel cheated because at school I was told that job opportunities would increase enormously if I had a degree and now four years later the choice of jobs is very limited".

In my own department, where some 65–70% of young graduates have obtained a higher degree, we see trained personnel leaving the country because of lack of opportunity. Although this provides some academic benefit to our department, it is almost impossible for these people to return to the UK. I know of two instances where our graduates could only afford to return if they were given professorships, because of the low salary scales in Britain.

In 1967 the Committee of Vice-Chancellors and Principals carried out a survey of the hours worked by university staff. An example of the hours/week was 54 for a vocational period, 63 for term-time and 80 for an examination period. University teachers do not receive remuneration for the vast amount of work which they take home. I wonder how many trade unions would tolerate a situation in which conditions of service are so loosely defined. Recently, Dr Alex Comfort commented on the situation in one research institute where "geriatric cases some of them, drawing fat salaries for years and doing absolutely nothing" created an impression of idleness among all the staff. Such situations are not unknown in universities and could be controlled

with a scheme of voluntary retirement before the official retiring age.

It might be considered relevant to design courses to meet the needs of the country but it would surely follow that relevant employment with a career structure should be available at the end of the course.

In 1968, however, a learned society formed a temporary committee for liaison between employers and teachers. The conflicting demands of employers were such that it was considered to be almost impossible to design a completely acceptable course for all employers. One must realise, therefore, that the demands of the employer should not dominate the discussion.

The increase in student-staff ratios from 8.4:1 to 10–11:1 is irrelevant when considering increased productivity and the number of staff to be retained in universities. The student-staff ratio or FTE (formal teaching equivalent) does not include the requirement for repetitive teaching to small aliquots of one large class, that is, the staff time involved to complete one course. I would suggest that increased expansion and productivity, if it is desirable, is a function of staff-student ratios, staff-time per student, space to accommodate larger classes, job opportunities, salaries, rationalisation of university courses, the needs of the country and continued investment in universities.

The last proposal to examine the balance between teaching and research in universities is very important. In view of the anticipated increase in time spent on teaching it seems that Lord Crowther-Hunt is proposing a reduction in research programmes. The subsequent effects of such a move would be disastrous. The move would not immediately affect the quality of teaching but would eventually affect the quality of universities. This would severely affect student populations and bright students would flee. There is a school of opinion which believes that the present government equates private education in the secondary field with universities in the tertiary field of education. Before Lord Crowther-Hunt and his colleagues in the Department of Education and Science run the bulldozer over the universities and produce comprehensive tertiary education they should consider the consequences.

D. E. S. STEWART-TULL

Microbiology Department,
University of Glasgow, UK

English and editorial boards

SIR,—Dr M. Charles (May 8) wonders what members of the editorial board of certain journals do, apart from collecting an annual fee and refereeing a paper or two. Speaking as a literary hack whose job it is to edit such papers so as to give them a veneer of literary and scientific competence, I question whether these august editorial names ever referee any of the papers submitted because (a) they would not be able to understand some of the appallingly ill-written stuff, (b) if they could understand it they would realise that much of it is not worth publishing.

Papers submitted in English by authors with another mother tongue are certainly often unintelligible, but it must be confessed that some native efforts from certain of our English universities and technical colleges run them pretty close. This is due to the recent proliferation of such institutes, with the result that anyone who is not actually moronic can now qualify for higher education. The effect is that people who in saner times would be happily and usefully employed behind the counter of the local grocer's now feel impelled to give the world the fruits of their intellectual labours.

J. C. ANDREWES

Cambridge, UK

Not worried

SIR,—I really rubbed my eyes at your contributor John Hall's statement that in Cambridge "The number taking chemistry is so small that professors are starting to worry about the security of their salaries ..." (*Nature*, May 22). The facts are precisely the reverse. The number of third year students opting for chemistry at Cambridge has risen steadily over the past three years and there is already a firm indication of 70 for next year's numbers (the final figure is usually above this initial assessment). The postgraduate situation here shows a similar robust health, particularly in organic chemistry, where it is clear from the shoal of applications that every available place will be filled. I can therefore reassure John Hall that he need not rattle a collecting box for Cambridge chemistry professors just yet.

R. A. RAPHAEL

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news and views

ALMOST six years have passed since the first manned landing and the return of lunar soils and rocks to the Earth. Six manned American and two automated Soviet samplings have amassed some hundreds of kilograms of lunar samples, many thousands of pictures and vast collections of instrumental data. The rocks and soils have been photographed, microphotographed, measured for almost every conceivable physical property, picked over, separated, melted, dissolved, and analysed for elements and minerals. What do we now know about the Moon after this first detailed look at another body in the Solar System, using combined space technology and laboratory analyses?

The meeting at the Royal Society attempted to answer this question, but inevitably it has raised many others.

History of the Moon

One major theme was the lunar time scale. An initial Moon-wide melting around 4.6 Gyr to 4.4 Gyr ago, to an undetermined depth, seems to have led to extensive chemical differentiation, producing the aluminium-rich lunar highlands underlain by a mantle rich in iron and magnesium. An intense asteroidal and meteorite bombardment then produced the dense cratering of the highlands and ended rather abruptly at 3.9 Gyr ago, possibly during a final burst of increased activity following some undetermined 'cataclysmic' event. The next phase from 3.9 Gyr to 3.2 Gyr consisted of partial melting of the lunar mantle at depth, producing volcanic lavas which spread out as a thin sheet over the rubble, filling the major impact basins. During the last 3.2 Gyr the lunar lithosphere has been too thick and impenetrable to permit these extrusions to continue in all but a minor way. Events in this period have been mainly restricted to occasional large impacts, producing craters such as Copernicus and Tycho, and a steady infall of minor amounts of meteoritic debris.

Although this broad outline is widely accepted a number of fundamental difficulties remain. J. Wasserburg of Caltech raised a number of questions in connection with the early differentiations and the later generation of mare basalts. Was the initial differentiation largely complete by 4.4 Gyr ago or did it extend in a continuous way down to the time of the mare basalt flows? The isotopic data

Probing the Moon

from Correspondents

The Royal Society held a discussion meeting on 9-12 June entitled "The Moon—A New Appraisal from Space Missions and Laboratory Analyses".

appear to favour the former view. Refined uranium-lead data indicate that the iron-rich mantle, from which the mare basalts were later generated, was formed 4.4 Gyr ago. This being the case, where in the Moon are the lead daughter products which were generated from 4.6 to 4.4 Gyr? The mechanism for remelting the mantle at later times to produce the mare basalts is also a major unsolved problem, and what is the significance of the apparent absence of mare basalt clasts from the highland breccias? J. Geiss (University of Berne) stressed the mounting evidence from Apollo 11 samples that at least for this site chemically distinct basalt extrusions occurred over a period of 300 Myr. The crater counts in this area of the Moon by G. Neukum (Max-Planck Institute, Heidelberg) support this view.

A current controversy over the precise significance of the ^{40}Ar - ^{39}Ar ages of highland breccias was aired by several speakers. G. Turner (University of Sheffield) interpreted the ages of the Massif boulders from Apollo 17 as indicating the time of the Serenitatis basin impact, a significant marker in the lunar stratigraphic sequence. This view was supported by trace element arguments by E. Anders (University of Chicago). In contrast, O. Schaeffer (State University of New York, Stony Brook) stressed arguments supporting a significantly older age of 4.28 Gyr for Serenitatis, and P. Horn (Max-Planck Institute, Heidelberg) emphasised the importance of smaller events in resetting the argon clock. The various interpretations of the highland ages carry important implications for the crater counts made by and discussed by G. Neukum. An old age for Serenitatis would probably be consistent with a steadily decreasing cratering rate for the early highland bombardment whereas the younger age

seems to require a late stage cataclysm, or dramatically increased cratering rate, at 4.0 Gyr.

Lunar petrology

The basalt lavas that flooded the mare basins from about 3.9 to 3.2 Gyr ago show evidence for a genetic relationship with both the feldspathic crust and the ultramafic mantle. Petrologists and geochemists spoke for and against the Taylor-Jakes model of two-stage melting to explain these relationships. G. M. Brown (University of Durham) showed how the model could explain the 4.6 Gyr model ages of the basalts, the europium anomalies, and the contrasted crystallisation ages and generation depths of the high- and low-titanium basalts, if a terrestrial-type cumulate model involving convection is examined. Some deficiencies in the model were exposed by S. Kessen (State University of New York, Stony Brook) who indicated the need for a titanium-rich magma just below the crust (65 km) and the role of basalt contamination by crustal rocks. A. E. Ringwood (University of Canberra) doubted that the Moon was totally melted deeper than about 200 km early in its history (as required by the two-stage melting model), and argued for a lunar mantle similar in composition and density to the Earth's. Also that existing models for the total Moon composition were too rich in CaO and Al_2O_3 to give mare basalts by partial melting. M. J. O'Hara (University of Edinburgh) referred to his experimental evidence in support of two revisions of lunar basalt origins. First, crystal sorting within the surface lava flows precludes the use of hand-specimens to predict the nature of the lunar interior. Second, the existence of highly feldspathic liquids is evidence for the original presence of water in lunar highland magmas. These discussions and the restraints imposed by the lithospheric rigidity history and crustal-thickness variation, from gravity determinations by S. J. Sjögren (California Institute of Technology), indicated that the whole question of the Moon's thermal history and magma generation is still an open one.

Lunar magnetism

The study of magnetic properties of lunar samples now seems to be geared to the most important goal of estimating the intensity of the ancient mag-

netic field. D. W. Collinson, A. Stephenson and S. K. Runcorn (University of Newcastle) presented data suggesting a trend of decreasing field, from 1.3 Gauss at 4 Gyr to about 0.05 Gauss at 3.2 Gyr, but there was little sign of any concurrence with this idea from the other speakers. M. Fuller (University of California, Santa Barbara) showed palaeointensity results in which there was no discernible trend although these were based on an approximate method. The interpretation of lunar magnetic anomalies is also at a very interesting stage; D. Strangway (University of Toronto) showed that some anomalies can be accounted for by a layer of horizontally magnetised material in the floor of some craters. On a more global scale P. J. Coleman (University of California, Los Angeles) showed that satellite observations give the upper limit to the lunar dipole moment as about 10^{19} Gauss cm³. This low value virtually rules out the original magnetisation of surface rocks in a uniform external field as shown by Runcorn and indicates an ancient field of internal origin—a very important constraint on the lunar history models.

Microscale processes

Micrometeorite impact is responsible for the observed particle size distribution within the soil and the formation of secondary particles (glassy welded aggregates), and may be the major mechanism of lateral transport of dust grains. But there was controversy as to whether micrometeorite particles travelling normally to the plane of the ecliptic have the same size distribution as particles in the plane of the ecliptic and as to whether the micrometeorite flux has varied with time. No evidence has been found for the deposition of impact-produced material although in μ m-size craters up to 50% of the material from the projectile is retained.

The major erosional effect at the Angstrom level is due to sputtering by the solar wind and estimates of the rate of mass wastage generally are in good agreement. The chemical changes which can be effected by solar proton bombardment were discussed at length by C. T. Pillinger (University of Bristol), R. M. Housley (North American Rockwell) and T. Gold (Cornell University), particularly the formation of free iron which has implications for carbon chemistry, magnetic properties and the albedo of the soil. Although rare gas systematics of the soils are now fairly well understood there is still no completely adequate explanation of the excess of ⁴⁰Ar found, as pointed out by P. Signer (Swiss Federal Institute, Zurich). Chemical elements such as carbon and nitrogen have been accepted as indicators of

solar wind bombardment. T. Kirsten (Max-Planck Institute, Heidelberg) reported that nitrogen seems to be the more effectively retained element since N/C ratios for the lunar soil are in excess of those measured for the Sun.

The conference closed with a look into the future by Noel Hinners, head of NASA's planetary programme. He pointed out that lunar research has proved to be an excellent prototype for exploration of the Solar System. The Moon also serves as a proving ground for the necessary space technology. Further manned expeditions are unlikely before the late 1980s when lunar bases will probably be set up. Meanwhile, around 80% of the returned lunar samples still await detailed analysis. Plans are going ahead for an unmanned lunar polar orbiter which would carry out detailed Moon-wide surveys of a number of important elements and physical phenomena. Several UK scientists are hoping to participate in this project.

Collaborating T and B cells

from Fritz H. Bach

RECOGNITION of antigen by lymphocytes of the immune system is the basis of the exquisite specificity of response characterising that arm of our bodily defence against foreign invaders. With the realisation that lymphocytes can be grossly divided into two classes, T (thymus dependent) and B (bone marrow derived) cells, and that both of these show great specificity in their response patterns, it was assumed by many that T cells must express specificity in a way similar to B cells. Our understanding that B cells carry immunoglobulins on their surfaces which are used as antigen recognition sites, or receptors, led logically to the possibility that T cells use the same set of molecules as receptors. This assumption has, during the last few years, been queried by many; now again there is immense controversy over whether T cells, like B cells, use immunoglobulins as their membrane receptors.

A possible clue relating to the genetic control of T-cell receptors came from the finding that genes of the major histocompatibility complex (MHC) control immune responsiveness to specific antigens. These immune response, or *Ir*, genes seemed to be expressed in T cells. Later experiments demonstrated that *Ir* genes could also be expressed in B cells.

With the appreciation of the distinction between T and B cells came evidence that these two cell types cooperate in antibody production by B cells—a phenomenon referred to as

T-B cell collaboration. For what is known as a T-dependent antigen, both T and B cells must recognise the antigen; the B-cell response (antibody production) is dependent on help from the T cell. The first suggestion that this T-cell help for B cells may come by way of a humoral factor presumably secreted by T cells came from work of Dutton and coworkers.

These two apparently separate lines of investigation involving T-cell factor(s) in T-B collaboration and the genetic control of the immune response were brought together by the observation in several laboratories that the T-cell product which facilitated B-cell response carried antigenic specificities determined by MHC genes; further that these genes mapped genetically with the *Ir* genes. The situation is best analysed in mouse. The *I* region of *H-2*, the MHC in mouse, includes the *Ir* loci; in addition antisera have been raised against *I* region products defining *Ia* (*I* region associated) antigens. It is with the anti-*Ia* antisera that the T cell product reacts. Since in the experimental systems of at least some laboratories the T-cell factor has antigenic specificity, it becomes a prime candidate for the T cell receptor. That the factor is not immunoglobulin is supported by several lines of investigation detailed by Munro and Taussig. (this issue of *Nature*, page 103).

The perplexing finding that *Ir* gene control is sometimes expressed in T cells and other times in B cells now seems to have a logical explanation. A number of groups (for references see the article by Munro and Taussig) now have evidence that two separate *Ir* genes linked to *H-2* are involved in the control of a given immune response. One of the two genes controls the ability of the T cell to produce the factor; the other appears to control a 'receptor' on the B cell which combines with the secreted T cell factor. Whereas the T cell factor clearly carries *Ia* antigenic determinant(s), the receptor on the B cell has so far only been shown to be associated with antigens defined by sera against both the *Ia* antigens and other *H-2* determinants although Munro and Taussig reasonably argue that the active moieties in these sera may also be anti-*Ia*.

The evidence provided by these authors and others is elegant in the synthesis they provide for the dual genetic control, the elucidation of the dichotomy between control of factor production versus a defect in factor acceptance and in the implications of these findings for cell collaboration. Although at least to some their model may seem awkward in certain minor details, especially in their attempt to explain the specificity of response

defect by limiting the responsiveness toward any given antigen to only some 'classes' of T cell receptors, it stimulates not only further experimental protocols in T-B collaboration but in several other systems. The importance of the Munro-Taussig work, with its demonstration of antigen specificity of the T-cell factor, the individual *Ir* control of T cell factor and B cell receptor for that factor, thus lies not only in the advance this provides for cellular immunology but also in the broader applicability of the model for cell cooperation which can now be studied at the molecular level.

In addition the two MHC *Ir* gene control of immune responsiveness is exciting to the geneticist. The MHC has often been referred to as a supergene as described by Fisher, that is, a group of linked genes that govern biologically interactive processes. During the past few years we have seen the elucidation of at least one such interacting system within the MHC where different antigens controlled by separate MHC genes (the SD and LD determinants) differentially activate

two separate populations of interacting T cells (T-T collaboration); an interaction which leads to the effective development of cytotoxic T lymphocytes. Munro and Taussig in fact speculate that their model for T-B collaboration may apply to T-T interactions. It may be that the antigens to which the separate T cell populations respond can also function as receptors (which may really be their major role) and that an even closer parallel than that envisioned by some will emerge between the LD-SD interaction and the T-B collaborating system.

Thus whereas many questions of key import remain (such as the possible participation of the variable part of the immunoglobulin molecule as a part of the T cell receptor, the question whether there are classes of T cell receptors which explain the specificity restrictions, and many others) the results and models generated by this group and others working in the same area are enormously exciting and provide a focus for the bringing together of several diverse experimental systems related to MHC genes.

Protein folding pathfinders

from Barry Robson

TRYING to discover how a globular protein folds up into its unique, biologically active conformation is not a task for the faint-hearted. The conformation of a protein is determined by hundreds of rotatable bonds, and hence its conformational energy can be described mathematically as a continuous surface with hundreds of dimensions. Through the hills and valleys of this conformational energy surface the molecule must weave its way in search of that single valley which represents the stable, biologically active conformation. A number of recent publications, some relating to experimental observations on folding proteins, and some to computer simulations of the folding process, give considerable insight into the most challenging and contentious aspects of the problem.

Pancreatic trypsin inhibitor is a relatively small protein of known three-dimensional structure and continues to be a popular model system. Creighton (*J. molec. Biol.*, **95**, 167; 1975) continues his experimental investigation of the folding of this molecule, again forcing the formation of disulphide bridges at selected intervals of time in order to covalently cross-link the backbone chain of the molecule and so trap intermediate conformational species. In this paper he directs his attention to the analysis of those intermediate species which have two such links, and observes that the various elements of the biologically active conformation must be assembled in a definite sequence. His findings therefore support the increasingly held belief that there is a unique and obligatory pathway for folding, and that the biologically active conformation is marked not so much by its own stability as the stability of the pathway leading to it.

Creighton's emphasis on a specific folding pathway seems at first glance inconsistent with his additional discovery that the protein in the early stages of folding has considerable conformational freedom in which many different conformations are built up. However, the initial population of the disordered, fully unfolded state contains an enormous variety of conformations which must be funnelled together into the first obligatory step at the start of the pathway proper. The initial intermediates therefore resemble the routes taken by the participants on their way to the official meeting place at the start of a sponsored walk.

Events at the beginning of the folding pathway have also been recently examined in ribonuclease A. Garel and Baldwin (*J. molec. Biol.*, **94**, 611; 1975)

New view of magnetic changes

from Peter J. Smith

THE magnetic field at the Earth's surface generally deviates from the north-south direction (declination), certain components of the field drift slowly westwards (secular variation), and from time to time the main field changes its orientation by 180° (field reversal). But although these phenomena are observable, directly or indirectly, their causes remain obscure. Declination, for example, is generally explained in terms of non-dipole components; but insofar as the origins of the non-dipole fields are uncertain, this merely shifts the obscurity one stage further along. And although it is usual to invoke eddy currents near the edge of the core, field perturbations which trigger greater changes in a homogeneous dynamo, and so on, such 'explanations' are generally intractable to rigorous analysis.

In sharp contrast, Steenbeck and Helmig (*Geophys. J.*, **41**, 237; 1975) have now put forward—tentatively—a quite different hypothesis which is not only amenable to mathematical treatment but is also able to link declination, secular variation and field reversal within a common theoretical development. The theory relates strictly to a simple model of a particular form of gyroscope—a rigid body free to rotate within a rotating hollow sphere and weakly

coupled to the sphere by isotropic friction. Steenbeck and Helmig propose, however, that the analysis may also be applicable to the Earth, with the solid core representing the rigid body of the theory.

In Earth terms, the theory predicts that varying irregularities in the magnetic field are a necessary consequence of a slow rotation of the solid core (gyroscope)—a rotation which is both possible and likely. The interaction between field and core arises basically because, being electrically conducting, the core should be penetrated by a part of the field which it then carries along. The axis of the core's rotation will lie in the Earth's equatorial plane and will rotate within it, thereby giving a westward drift of varying velocity (although eastward drift could also arise under certain circumstances). But the rotational state producing the drift ultimately becomes unstable; and the solid core then tips over relatively rapidly, possibly initiating a total field reversal.

There are several problems, chief of which is that mass movements in the fluid outer core will lead to friction which is neither weak nor isotropic. But although this may alter the quantitative argument, it should not invalidate the principle.

have shown that the thermally unfolded state of this protein is an equilibrium mixture of at least two species, one which initiates rapid folding and one which initiates slow folding. In an extension of this study (*J. molec. Biol.*, **94**, 621; 1975) the authors confirm and explore a physical difference between these species. Kinetic analysis shows that the fast-folding species is an intermediate on the folding pathway from the slow-folding species, but, unlike most intermediates found in proteins so far, it represents a considerable fraction of the initial unfolded state. Although the initial unfolded state prepared by heat denaturation of the native protein may not resemble an initial state prepared in other ways, the study does highlight the possibility that the unfolded state itself may not be one of homogeneous disorder.

In conjunction with Creighton's view this would suggest a picture in which our sponsored walkers converge on the official starting point not from a wide variety of different home addresses, but from a limited number of densely populated areas.

The relatively small size of pancreatic trypsin inhibitor makes it an equally popular system with those who would simulate protein folding by computer. After all, proteins contain thousands of atoms, while there are very much smaller molecules at which a theoretical chemist would sensibly balk. Even so, Burgess and Scheraga (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1221; 1975) have produced what at first reading appears an uncharacteristically melancholy document demonstrating that by existing algorithms it is impossible to simulate the folding of trypsin inhibitor.

Their conclusion might appear to contrast with that of Levitt and Warshel (*Nature*, **253**, 694; 1975); but it would not be entirely justifiable to compare these conclusions since they relate to two fundamentally different methods with different immediate aims. Whereas the Levitt-Warshel approach is to reduce the complexity of the energy surface by simplifying the representation of a protein molecule, Burgess and Scheraga favour retaining a more realistic representation of a protein while reducing the computing time by other methods. In particular, they favour starting the simulated folding process from conformations which they hope are already fairly close to the biologically active conformation, at least as far as the conformation of each amino acid residue in the protein backbone is concerned. A variety of algorithms for predicting such starting structures already exists, most of them based on statistical analysis of amino acid residues in proteins of known sequence and conformation. Burgess

and Scheraga find all these predictions wanting as practical starting structures because the ambiguities and inaccuracies currently inherent in the algorithms which produced them must lead to major ambiguities and uncertainties concerning the conformation of the protein as a whole.

The theoretician seeking to fold proteins inside the computer therefore seems to face a dilemma of either using a simplified representation of a protein molecule, and so study folding across a possibly unrealistic energy surface, or of using a more realistic representation, and sending the folding process down the wrong pathway. In practice, no one has proposed using only one of these approaches and it is reasonable to expect that the ultimate practical and repeatable method will utilise elements of both. In any case, current understanding of the folding of proteins suggests that the starting configurations considered by Burgess and Scheraga are more than a convenient artifice. Creighton's experimental work supports the idea that fairly early steps in the folding process are restricted to a limited number of pathways by thermodynamically stable intermediates. If predicted starting conformations can be identified with such intermediates then they represent structures which ought to appear early in any realistic folding simulation. Ultimately successful computer programs using statistical prediction algorithms to select starting conformations would then merely resemble elderly sponsored walkers taking advantage of a handicap system which allows them to join the walk at a later stage without sacrificing any sense of communal achievement.

Fortunately, the prediction of suitable starting conformations is only one of the methods by which computing time can be reduced. In particular, Hagler and Robson (*Specialist Periodical Reports*, **6**, 207; The Chemical Society, 1975) have reviewed a class of procedures which, by analogy with methods used for solving complex problems in other fields, may be termed 'heuristic'. But they used the term more specifically for procedures which obtain additional information from outside the conformational energy surface. Up till now, such heuristic procedures have generally been confined to the prediction of starting conformations or the imposition of constraints on the possible final folded structures, but recently Nagano and Hasegawa (*J. molec. Biol.*, **94**, 257; 1975) have made use of a penalty function which applies throughout the simulated folding. This penalty function includes estimates of the interaction between residues far apart in the protein backbone, but brought close together in three dimensional space by the folding of the protein. These

estimates are dependent on the conformation of the interacting residues and were derived by previous tests on proteins of known sequence and conformation. The use of penalty functions is in the best tradition of heuristic programming in its widest sense, and will probably prove very useful. But Nagano and Hasegawa caution that "It would be premature to discuss [whether the penalty function as described] is realistic or useful". Clearly this kind of approach is still in its infancy.

Finding the pathways by which proteins fold taxes the ingenuity and resources of experimentalists and theoreticians alike. In order to communicate their ideas, protein folding pathfinders have developed or borrowed concepts and terminology which frequently confound other life scientists. It would be regrettable if this obscured the prevailing pioneering spirit, for what may at this moment in time be a sponsored walk for a protein molecule is an exploration of uncharted territory for the scientist.

Getting more CO₂ into food

from Israel Zelitch

'PHOTORESPIRATION' finally became a familiar word to the top Wall Street executives who read their June issues of *Fortune* or *Business Week* magazine. Articles unexpectedly cropped up there describing the potential importance of slowing photorespiration for increasing food production. At the same time about two hundred scientists (a fairly even mixture of plant physiologists, biochemists, plant breeders, and agronomists) gathered in Madison, Wisconsin to attend the Fifth Steenbock Symposium held from June 9 to 11 at the University of Wisconsin.

The subject of the symposium was billed as CO₂ metabolism and productivity of plants. A number of topics relating to photosynthesis and productivity were talked about, but nine of the twenty-five scheduled talks and most of the questions and discussions in the conference hall seemed to deal with the background and latest work on photorespiration. The greatest controversy was generated by the difference in the views expressed by some leading investigators about the biochemical mechanism for the synthesis of glycolic acid, the chemically simple but enigmatic substrate of photorespiration.

The symposium was organised by R. H. Burris (University of Wisconsin), whose laboratory published some of the first plant biochemistry relating to glycolic acid in 1949, and C. C. Black (University of Georgia). Talks by crop physiologists and plant breeders were

interspersed with papers of a more biochemical nature.

The programme covered recent progress in C_3 -metabolism (species with fast photorespiration and slow net photosynthesis), C_4 -metabolism (species with slow photorespiration and fast net photosynthesis), and crassulacean acid metabolism (a combination of C_4 - and C_3 -biochemistry that occurs mainly in species normally found in arid habitats).

Ribulose diphosphate carboxylase is the enzyme mainly responsible for fixing CO_2 during photosynthesis, the product of the reaction being two molecules of phosphoglyceric acid. W. L. Ogren (University of Illinois) and N. E. Tolbert (Michigan State University) presented the evidence that this enzyme has a dual activity since it also catalyses a reaction between the substrate (ribulose diphosphate) and O_2 . The products of the 'oxygenase' reaction are phosphoglycolic acid (a precursor of glycolic acid) and phosphoglyceric acid (the same product formed by the carboxylation reaction). Since O_2 competes with CO_2 for the isolated enzyme in a manner reminiscent of the inhibition of CO_2 assimilation by O_2 in leaves (the Warburg effect), they argue that the 'oxygenase' reaction accounts for all of the glycolic acid produced in leaves and hence all of the photorespiration. Ogren, R. G. Jensen (University of Arizona), and R. C. Chollet (duPont Co.) described biochemical schemes containing carbon balances that they stated could account for glycolic acid synthesis and photorespiration. These schemes assume that all of the glycolic acid comes from the 'oxygenase' reaction and that no more than about 20% of the carbon fixed during photosynthesis is released as photorespiratory CO_2 .

Jensen added that he thought there might be other biochemical mechanisms for glycolic acid synthesis. A critical listener noted that none of the schemes account for the well documented observation that photorespiration often consumes at least 50%, and not 20%, of the CO_2 fixed during photosynthesis. Another omission seems to be that the stoichiometry of the products of the 'oxygenase' reaction, on which the above schemes depend, has never been presented. A further weakness of the argument is that the rates of glycolic acid formation by the 'oxygenase' activity in normal air with Jensen's lysed chloroplast preparations are only about 10% as fast as rates occurring in leaves.

There was a strong difference of opinion over whether the ratio of the 'oxygenase' to carboxylase activity is a constant. Tolbert showed that certain metabolites, such as ribose 5-phosphate, could inhibit the 'oxygenase' and not

the carboxylase activity and suggested that metabolites could affect the balance between net CO_2 assimilation and photorespiration. C. C. Black stated that this ratio of activities could vary by as much as 10-fold in leaf extracts from different species. During the course of a special discussion session largely devoted to problems relating to the assay of the 'oxygenase' activity, it became apparent that many factors affect the assay including whether or not the enzyme has had prior contact with bicarbonate.

The C_4 crop species (maize, sugarcane, sorghum) generally have much higher rates of net photosynthesis and productivity than the C_3 -species. M. D. Hatch (CSIRO, Canberra) further elaborated the hypothesis that these species are able to raise the CO_2 concentration in their leaf bundle sheath cells by transporting malic acid (or other C_4 compounds) into these cells and then decarboxylating it. The resulting higher CO_2 level in the bundle sheath cells inhibits glycolic acid synthesis and photorespiration and promotes a speedup of CO_2 fixation by enzymes of the pentose phosphate cycle. The CO_2 , he believes, does not have much of an opportunity to diffuse out because it is fixed so rapidly.

Painstaking cytogenetic work on the results of several generations of crosses between a C_4 - and a C_3 -species of *Atriplex* was described by O. Björkman (Carnegie Institution, Palo Alto). The hybrids produced, however, had poorer rates of net photosynthesis than either of the parental types, and he concluded that it is extremely unlikely that superior hybrids will be obtained by conventional interspecific crossing.

Plant breeders who are anxious to incorporate superior photosynthesis into their plants in order to obtain higher yields have had many frustrations and misgivings. This was clearly illustrated in the talk by D. H. Wallace (Cornell University) who emphasised that there are numerous systems and interactions concerned with plant productivity besides photosynthesis. He argued that changing only one component of the system, such as photosynthesis, would probably not increase the yield say of dry beans. A member of the audience pointed out that plant breeders have not yet had the opportunity to move a characteristic such as superior photosynthesis into different genetic backgrounds in order to make a proper evaluation. A listener also remarked that increasing net photosynthesis by raising the CO_2 concentration in a closed system would surely increase the yield of dry beans, and one must therefore assume that if large increases in net photosynthesis could be obtained in normal air it would be similarly beneficial.

Many participants felt that more opportunities such as this symposium should be created to permit research workers from different disciplines to share information about plant productivity. It appeared to me that the world food problem has finally touched many scientists who might previously have been more aloof from problems existing in the real world.

Assembly of regular viruses

from a Correspondent

The Discussion Meeting arranged by A. Klug and P. J. G. Butler (MRC Molecular Biology Laboratory, Cambridge) for the Royal Society on June 18 and 19 was held to review the more recent work on the spontaneous assembly of viruses, with particular reference to the 'regular' viruses.

As a group viruses have been found to have surprisingly few similarities other than those dictated by size, pathogenicity and the available building materials. Constrained to have their hereditary material as some kind of nucleic acid they may have RNA or DNA (but so far not both) in any of many forms, single stranded, double stranded, circular, in several pieces and so on. Not surprisingly the associated coat structures are equally variable, so that the discussion proved to be more of an analysis than a synthesis.

Although the self-assembly of the tobacco mosaic virus has been recognised for twenty years its mechanism is not yet fully understood, as was made clear by K. E. Richards (Institut de Biologie Moléculaire et Cellulaire, Strasbourg) and P. J. G. Butler and D. E. G. Zimmern (both MRC Laboratory of Molecular Biology, Cambridge). A method for finding the 'recognition site' of the viral RNA is the selective coating of its fragments, a method previously employed by J. G. Atabekov (Moscow State University) who yet again was prevented from attending a meeting in this country. Rather unexpectedly, these fragments have been found by Richards and his colleagues to contain the coding sequences for the coat proteins, which are known from other evidence not to be in the recognition site region.

In the case of one of the small spherical viruses, brome mosaic virus, which is held together by hydrogen bonds between carboxyl groups and by protein-RNA interaction, the coat was shown by B. Jacrot (Institut Max Von Laue-Paul Langevin, Grenoble)

to be assembled from protein subunits existing in a metastable state. He also presented data on the protein-RNA locations in this virus from small angle neutron diffraction in D_2O-H_2O mixtures, a technique with considerable potential. The requirement for metastable intermediates was also put forward by J. King (Massachusetts Institute of Technology) to explain the complex but orderly assembly of the tail of the T4 bacteriophage. Several speakers discussed the details of the band assembly of this and other bacteriophages. An interesting sidelight is the re-use of components necessary for the assembly but not incorporated in the mature virus.

Other speakers dealt with various small viruses including the flexuous plant viruses, one of which, potato X virus, has been reassembled *in vitro*, and spherical viruses, like the turnip yellow mosaic virus, the shell of which is stable in the absence of the nucleic acid, does not rely upon carboxyl hydrogen bonding for its stability and has not yet proved completely amenable to efforts to reconstitute it.

Antigen receptors on T lymphocytes: a solution in sight?

from Melvyn Greaves

A symposium on "Membrane Receptors of Lymphocytes", organised by M. Seligmann, F. Kourilsky and J.-L. Preud'homme, was held in Paris on May 22-24.

THE molecular nature of the cell surface receptor for antigen on T (thymus-derived) lymphocytes has provided one of the central controversies of immunology over the last five or six years. The meeting in Paris produced a major step forward in the resolution of this problem and the closely related question of immune response gene function. Though the meeting included papers on other lymphocyte surface structures including IgD, F_c receptors, C3 receptors, β_2 microglobulin and Ia antigens, it was the T cell receptor question which once again held the centre of the stage.

Substantial though equivocal evidence had accumulated earlier that the T cell receptor was an immunoglobulin-like molecule—similar if not identical to that on B ('bursa-equivalent' derived) lymphocytes and in serum. The evidence was based partly on the apparent diversity and specificity of T cell responses and also on the capacity of some anti-immunoglobulin sera to block various T-cell activities. But since 1971

three groups of data have considerably influenced research on this question.

First, attempts to chemically identify T cell surface immunoglobulin using lactoperoxidase iodination methods (labelling followed by extraction immunoprecipitation and polyacrylamide gel analysis) have been extensively reported (*Transplant Rev.*, **14**; 1973). J. J. Marchalonis and colleagues from the Walter and Eliza Hall Institute in Melbourne have maintained that they can reproducibly find considerable quantities of IgM on T cells whereas J. Uhr and E. Vitetta (University of Texas, Dallas), R. M. E. Parkhouse and E. Abney (National Institute for Medical Research, London), B. Lisowska-Bernstein and P. Vasalli (University of Geneva) have failed to reproduce this result. Discussion of this discrepancy, centering on technical details, though a regular feature of international conferences, has failed to provide a realistic means of settling the dispute.

Second, the *Ir* ('immune response') gene phenomenon has mushroomed to become a dominant theme of immunology. B. Benacerraf, H. McDevitt and M. Simonson in particular have proclaimed that the T cell receptor was a product of genes linked to the major histocompatibility locus of the species (*H-2* in mice, *HL-A* in man). This speculation was based to a large extent on the view that *Ir* genes were expressed principally at the level of T cells, and on the ability of certain alloantisera directed towards *H-2* or equivalent loci to suppress T cell activity. Third, the discovery that β_2 microglobulin had considerable sequence homology with IgG (see Raff, *Nature*, **254**, 287; 1975) and yet was found associated with *HL-A* and *H-2* on cell surfaces opened up a different philosophical approach to the T cell receptor question. The characteristics of β_2 microglobulin imply that a phylogenetic relationship may exist between immunoglobulin and products of the major histocompatibility locus although there is in man no genetic linkage between genes coding for *HL-A*, β_2 and immunoglobulin. An evolutionary relationship between *HL-A*, *H-2* and immunoglobulins is further reinforced by recent studies suggesting that the former have an overall tetrameric structure and compact domains that are strikingly similar to those in immunoglobulin molecules (Peterson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1612; 1975).

This crucial new information supports something of a compromise solution to the T cell receptor mystery since it could be both Ig-like and *H-2* (in the mouse)-like! Several authors have suggested for example that it might have an immunoglobulin-like V

region and a constant region coded for by genes linked to the major histocompatibility locus (see for example Gally and Edelman, *A. Rev. Genet.*, **6**, 1; 1972).

Two papers on this problem in particular generated a great deal of interest. Hans Wigzell (Uppsala University, Sweden) reported on work with Hans Binz which provided very compelling evidence that the same idiotype or variable region gene product can be found on both B and T lymphocytes. The experimental design they used was based on a series of experiments reported several years ago by H. Ramseier (*Transplant Rev.*, **10**, 57; 1972); these were, in retrospect, outstanding in the extent to which they were ignored or disbelieved by immunologists. In brief, if $(P \times Q)F_1$ animals (rats in this case) are injected with parental P-type lymphocytes then clones of P cells which are specific for Q antigens will proliferate and secrete antibody (if they are B lymphocytes) or become cytotoxic and release 'lymphokines' (if they are T cells). The recognition in this situation might, on genetic grounds, be thought to be entirely unilateral (only $P \rightarrow Q$); but P cells do have something which $(P \times Q)F_1$ cells can recognise and presumably lack themselves. This is the idiotype of the anti-Q antibody and the corresponding receptors on the P anti-Q T and B lymphocytes. $(P \times Q)F_1$ rats can indeed be shown to make antibody to this idiotype(s) either in response to the injection of unseparated P cells (or relatively pure T cells from P rats) or to the infusion of P anti-Q serum antibodies. The antibodies so raised react with P anti-Q antibodies, B cell surfaces (about 1% of B cells) and T cell surfaces (about 7% of cells). All the activity against T cells can be absorbed out by either B lymphocytes or P anti-Q antibodies. Preliminary evidence suggests that the idiotypic determinant on B cells was associated with a molecule the same size as 7S IgG (molecular weight 120,000) whereas the T cell product had a molecular weight around 35,000. The latter was non-reducible and therefore possibly a single polypeptide chain. But it cannot at this stage be ruled out that this is an 'incomplete' receptor which is non-covalently bound to other subunits or chains when *in situ* on the cell surface.

Klaus Eichmann (University of Cologne) presented what appears to be a strikingly similar story. He had raised antibodies in guinea pigs to the idiotype(s) of anti-group A streptococci antibodies produced by cloned (*in vivo*) mouse B cells. Although direct binding to T and B lymphocytes was not tested these antisera had very intriguing effects when injected into mice. Anti-idiotype of the IgG2 class suppressed both T ('helper') and B (antibody-forming cell

precursor) responses; whereas IgG1 antibodies stimulated anti-streptococci B clones (at high concentrations) or both B and T anti-streptococci clones (at low concentrations).

These two sets of observations strongly suggest that T cell clones have on their surface a molecular structure—presumably the antigen receptor—which bears idiotypic antigenicity similar if not identical to that of B cell receptors and B cell-derived serum antibodies. Although unequivocal evidence for the biosynthesis of this determinant by T cells is not yet available these results clearly imply that T and B lymphocytes are equipped with a similar 'immunoglobulin' V gene pool and may have at least some identical genes.

Presumably the amino-terminal end of the T cell receptor polypeptide contains both the idiotype determinant and the antigen combining site. The crucial question now is the nature of the remainder of the molecule. Is it an *I* region gene product with invariant peptide sequences and domains analogous to those coded by immunoglobulin C (constant) genes? If this is the case and V genes are identical in T and B cells we have the problem of how unlinked V and C (*I* region) gene products become associated, since in B cells the gene pairs are linked and association is believed to occur at the transcriptional level. Alternatively, could the T cell receptor have an immunoglobulin (of a new class?) C region after all? J. Marchalonis and N. Warner suggested that this latter view was strongly supported by their evidence that almost all T-cell derived neoplasms synthesise immunoglobulins. Significantly, with respect to other discriminating lymphocyte cell surface products (for example θ , Ly antigens) these lymphomas maintain a strict T cell phenotype.

The former view is however probably the popular choice and presumably immunochemical analysis of the Wigzell-Binz idiotypically defined T cell product should soon resolve the issue. This interpretation is also supported indirectly by an elegant series of experiments by M. Taussig and A. Munro (see this issue of *Nature*, page 103). Their important and provocative results suggested that activated T cells released an *I* region coded glycoprotein (molecular weight about 50,000) which facilitates the B cell antibody response. The 'factor' probably has antigen specificity although this critical point has not been clearly established. Mice of different strains which did not respond to (T,G)-A-L may be deficient in T cells producing the factor, in B cells capable of 'receiving' the factor, or both! An observation of paramount importance was the result of crossing

T cell 'deficient' mice with B cell deficient mice; the F_1 offspring were responders presumably by complementation of T and B cell *I* region-controlled immunocompetence.

A similar *I* region complementation effect was reported by B. Benacerraf (Harvard Medical School) and was in fact reported with minimal effect by E. Rude and E. Gunther at the Second International Congress of Immunology last year (*Progress in Immunology II*, vol. 2, 223; Associated Scientific, 1974). The main conclusion emerging from the work of Taussig and Munro is that the *I* region may contain structural genes coding for cell surface glycoproteins (with Ia antigenicity) on both T and B cells. These structures are critically involved in antigen recognition and collaborative interactions between T cells, B cells and possibly macrophages as well. If this is correct then the *I* region contains genes which not only code for antigen binding sites but also for a 'mutual' recognition system (that is, of T cell Ia molecules by B cell surface Ia molecules).

It seems reasonable to suppose that the molecule studied by Taussig and Munro might be the same as the idiotype-bearing structure identified by Wigzell and Binz, although H. McDevitt (Stanford University) reported that anti-Ia sera could not block (T,G)-A-L antigen binding by T (or B) lymphocytes. Taken together however these results certainly created the strong impression that the T cell receptor mystery may be soon solved. In the meantime, some healthy scepticism is probably called for. T cell populations are clearly very heterogeneous and different types of receptors might exist on subsets programmed for distinct functions. The role of macrophage surface structures in the genetic control of T+B responses merits further consideration. The precise antigen specificity and diversity of putative T cell receptors requires clearer definition as do both their immunochemistry and the cellular site of biosynthesis and uptake.



A hundred years ago

In connection with the calamitous floods around Toulouse, on the 25th June a singular phenomenon was observed at Clermont-sur-Lanquet. The whole of the earth on the slope of a mountain was moved bodily, a shepherd's house being transported uninjured to a distance.

from *Nature*, 12, 220; July 15, 1875.

Calcium transport in contraction and secretion

from N. M. Green

The growing interest in the role of Ca^{2+} in a variety of intracellular control mechanisms was reflected in an international symposium on Ca^{2+} transport (Bressanone, May 10–16, 1975) which was devoted to establishing contact between workers in the field of muscular contraction, where the effects of Ca^{2+} are relatively well understood, with those who work on a variety of secretory systems. This report considers only a few of the fifty papers that were presented, emphasising those aspects which may lead to fruitful interactions.

ONE of the main technical problems in the way of a more precise definition of the role of Ca^{2+} in secretory systems is the difficulty of measuring changes in submicromolar, cytoplasmic Ca^{2+} concentrations in the presence of intracellular and extracellular reservoirs where concentrations are increased a thousand-fold. J. Meldolesi (University of Milan) described the analyses of subcellular fractions from pancreas. Interpretation was difficult because of the rapid exchange of Ca^{2+} between compartments after cellular disruption. However, there was an interesting observation of a large non-exchanging reservoir of Ca^{2+} in the zymogen granule fraction. A similar high Ca^{2+} content of secretory granules was observed in pancreatic islet tissue (W. J. Malaisse, University of Brussels) and in parotid gland (Z. Selinger, Hebrew University, Jerusalem). J. L. Borowitz (Purdue University) suggested that uptake of Ca^{2+} by the granules in adrenal medulla might play an auxiliary role in the post-secretory recovery process. A promising new approach to the problem of intracellular Ca^{2+} distribution is that of X-ray microprobe analysis which can detect changes of local concentrations of Ca^{2+} within organelles at a resolution of 50–100 nm, provided that local concentrations, which can be enhanced by micro-incineration, are high enough (T. A. Hall, Cambridge). A study (with R. Yarom, Hebrew University, Jerusalem) of cardiac muscle revealed unexpectedly high levels in cell nuclei, associated particularly with heterochromatin, suggesting that serious consideration should be given to the nucleus as a reservoir of intracellular Ca^{2+} . M. J. Berridge (University of Cambridge) described another application of the technique to show that

mitochondria of insect (*Calliphora*) salivary gland release their Ca^{2+} following stimulation of secretion by serotonin. Unfortunately, cytoplasmic Ca^{2+} levels are far below the sensitivity of this method, which anyway cannot distinguish free from bound Ca^{2+} . The most suitable technique at present available for measuring free Ca^{2+} is the use of aequorin and it has not yet proved possible to introduce this protein into secretory cells.

Electrophysiological results

Extensive published work by Katz and Miledi has established the existence of time- and potential-dependent channels for Ca^{2+} in synaptic and neuromuscular junctions. The application of similar electrophysiological techniques to secretory systems, partly aimed at testing the wider applicability of these concepts, was described by several groups. E. K. Matthews (University of Cambridge) showed a close correlation between secretion of insulin and spike potentials induced in pancreatic islet cells by D-glucose or by current injection. Since this electrical response depended entirely on the presence of external Ca^{2+} it was possible to calculate from it the magnitude of the Ca^{2+} influx and consequent changes in local internal concentration of the order of 1–2 μM . Glycolytic metabolism was essential to the response implying that the change in permeability was caused by a metabolite rather than by glucose itself. In contrast potential changes in exocrine pancreas induced by acetylcholine or pancreozymin (O. H. Petersen, University of Copenhagen) proved to be Na^+ rather than Ca^{2+} dependent implying that the Ca^{2+} known to be required in the activation of secretion was derived from internal stores.

T. J. Rink and P. F. Baker (University of Cambridge) showed that K^+ depolarisation of the adrenal medulla gave rise to a time dependent influx of Ca^{2+} which decayed with the half life of 45 s, very much slower than the corresponding effects in nerve and muscle. J. J. Dreifuss and J. J. Nordmann (University of Geneva) measured neurohypophysial influx and efflux of $^{45}\text{Ca}^{2+}$ following K^+ depolarisation. In this system, as in nerve axons, the Ca^{2+} efflux associated with the recovery phase appears to be driven by Na^+ influx rather than being directly coupled to ATP hydrolysis as in muscle. The Ca^{2+} influx was blocked competitively by micromolar concentrations of D.600 (methoxyverapamil). The use of this drug as a Ca^{2+} antagonist follows from the extensive work of A. Fleckenstein (University of Freiburg) who discussed its action on cardiac and on smooth muscle. Its use provided one of the few unifying features of the diverse secretory systems described at

the meeting. Almost all those which required external Ca^{2+} for activation (nerve terminals, neurohypophysis, cardiac muscle, adrenal medulla, endocrine pancreas, insect salivary gland, but not the parotid gland) were inactivated by D.600. It will be of interest to know how far it can be used to characterise molecular species involved in Ca^{2+} conductance. Unfortunately, it has other pharmacological actions as a reserpine analogue. Moreover, N. A. Thorn (University of Copenhagen) reported that in the neurohypophysis it counteracts activation by the Ca^{2+} ionophore A23187, possibly by non-specific complex formation between lipid-soluble amine and carboxylic acid.

The complex interplay between fluxes of Na^+ and Ca^{2+} was considered further in several contributions. E. Carafoli (Zurich Institute of Technology) showed that in the absence of permeant anions, Ca^{2+} taken up by isolated heart mitochondria could be released by low (50 mM) Na^+ . However, Thorn showed that under similar conditions the uptake of Ca^{2+} was also inhibited, throwing some doubt on the interpretation of this result. The lack of a defined physiological mechanism for bringing about release of Ca^{2+} from mitochondria makes it difficult to evaluate their role in controlling cytoplasmic concentration, especially since in the long term, as was emphasised by Rink, most of the Ca^{2+} that enters through the plasma membrane must leave by the same route. The results of A. B. Borle (University of Pittsburgh) suggesting that cyclic AMP stimulates Ca^{2+} release from isolated mitochondria have not so far proved repeatable. However, Berridge provided evidence for a similar control mechanism to account for his observed release of mitochondrial Ca^{2+} in insect salivary gland. Borle emphasised that erroneous conclusions about changes in Ca^{2+} concentrations can easily be drawn from isotopic flux measurements if there is no information about changes in pool size which can be very large.

The actions of cyclic nucleotides in contractile systems are relatively well understood on the biochemical level although the physiological significance is not always clear. S. V. Perry (University of Birmingham) described how the effects of Ca^{2+} in releasing actomyosin from inhibition by the troponin complex are modulated by phosphorylation of the troponin components I and T by either a cyclic AMP dependent protein kinase or by phosphorylase kinase. The phosphorylated amino acids have now been located in the sequences of the troponin components, but the way in which phosphorylation and dephosphorylation control sensitivity to Ca^{2+} remains obscure. Cyclic AMP has

a more clearly defined role in controlling the activity of cardiac muscle in response to adrenaline. A. M. Katz (Mount Sinai Hospital) showed that it could enhance the rate of Ca^{2+} uptake by the sarcoplasmic reticulum by activating the phosphorylation of a membrane protein (molecular weight 22,000) by a protein kinase. This phosphorylation was correlated with an enhanced relaxation rate.

It is likely that one direct relation between contraction and secretion will eventually be found at the level of the contractile process itself, since there is a plausible assumption in the field, though little direct evidence, that expulsion of secretory granules is powered by Ca^{2+} -activated actomyosin within the cell. Surprisingly, little was said about this aspect of the relationship, although several interesting papers were given summarising current knowledge in the field of troponin-tropomyosin-actin interactions and the dependence of their association on Ca^{2+} concentration. S. Hitchcock and J. Kendrick-Jones (University of Cambridge) found no functional relation between carp parvalbumins or myosin light chains and the Ca^{2+} -binding troponin component (C) in spite of significant structural homologies, discussed by R. Kretsinger (University of Virginia). Their work on the Ca^{2+} -dependent regulatory properties of myosin light chains in scallop muscle as well as the differences in mode of action of troponins from cardiac and smooth muscle described by S. Ebashi (University of Tokyo), emphasise that control of cellular actomyosin is likely to differ significantly from that of skeletal muscle.

Although initiation of relaxation in skeletal muscle by the sarcoplasmic reticulum is well understood, our knowledge of the mode of control of Ca^{2+} influx in muscle appears little further advanced than it is in secretory systems. The once favoured regenerative release of Ca^{2+} from sarcoplasmic reticulum by local high Ca^{2+} was re-evaluated by M. Endo (University of Tohoku), who showed that unphysiologically high Ca^{2+} concentrations were required and that the process was inhibited by procaine which does not affect the physiological, depolarisation-induced release. It would be of interest to know if the Ca^{2+} channels involved resemble those of nerve, heart muscle and secretory tissue in their susceptibility to the action of D.600.

The meeting usefully defined several borderline areas where more work could profitably be done, as well as emphasising the relation of contraction and secretion to more general intracellular control processes involving Ca^{2+} and cyclic nucleotides which were not directly considered.

review article

A hundred years of controversy over sunspots and weather

A. J. Meadows*

After a century of accumulating correlations on the activity of sunspots and atmospheric phenomena around the Earth, we still have no clear idea what exactly the relationships are or what they mean. Much of what is being researched now was already under discussion in the nineteenth century. This article is based on the author's recent Norman Lockyer Lecture at the University of Exeter.

ALTHOUGH the appearance and disappearance of sunspots was one of the first discoveries made with the newly invented telescope in the early seventeenth century, detailed studies with reasonably powerful telescopes had to await the resurgence of interest in solar studies that characterised the latter part of the eighteenth century. One factor in this renewed interest was the possible effect of solar changes on the Earth. William Herschel¹ carried out a detailed study of the solar surface during the period 1795–1800, and, having outlined his results, commented: "Since experience has already convinced us, that our seasons are sometimes very severe, and at other times very mild, it remains only to be considered whether we should ascribe this difference immediately to a more or less copious emission of the solar beams."

He then attempted to find some systematic variation in terrestrial weather that could be linked with changes on the Sun. The only relevant long period data available were some statistics on wheat prices collected by Adam Smith in preparing his book on *The Wealth of Nations*. Herschel assumed these would reflect the average weather conditions in each year, and attributed the fluctuations they showed to solar variation.

This initiative was not followed up for several decades. Herschel's emphasis on the physical properties of celestial objects, although not unique, was hardly characteristic of the first half of the nineteenth century. It was only in the 1860s that the new science of astrophysics became firmly established, and the possibility of a relationship between solar change and terrestrial weather once again came to the fore. By this time, two important observational advances had been made. First, Schwabe in Germany had pointed out that the number of spots visible on the Sun's surface rose and fell in a regular manner with a period of about ten years. Although he announced this result in the early 1840s, it was the early 1850s before it became generally known. Also during the early 1850s, it was found that the frequency of magnetic storms varied with the same period as the sunspots. Thus, by the 1860s, astronomers knew two things that William Herschel did not: that some changes of the solar surface occurred in a cyclical fashion, and that one indisputable relationship existed between solar and terrestrial changes. The latter encouraged the hope that further relationships might be found: the former suggested the way in which they might be sought.

Lockyer in 1872 asked the readers of *Nature*, "why it is that meteorologists, state endowed or otherwise, have, as a rule, been content to grope their way in the dark, and not only not seek to find, but persistently refuse the clue, which, if followed, would bring them into the light of day". He then explained what this clue was: "Surely in meteorology as in astronomy, the thing to hunt down is a cycle."

The problem from the beginning was that terrestrial weather obviously underwent major fluctuations from day to day. Thus, if cyclical effects extending over several years were to be discovered, it was either necessary to find a clear-cut meteorological phenomenon that showed minimum short-period fluctuations, or to take ordinary meteorological phenomena and look for systematic trends by averaging over large numbers of observations. Several people, Lockyer among them, saw the monsoons in the Indian Ocean as the sort of clear-cut phenomenon that should readily show the effects of solar change. Lockyer chanced on this idea: Meldrum, who was in charge of meteorological observations on Mauritius, approached the problem a good deal more systematically. He examined the number, size and duration of cyclones occurring in the Indian Ocean during the period 1847–73, and showed that all these variables were enhanced at solar maximum compared with minimum. He also ingeniously correlated the sunspot cycle with the number of vessels putting into Mauritius for repair after damage caused by heavy seas. His extracts³ from the official port records make livelier reading than most sets of statistics, for example: "December 16–17, 1867. Ship 'Berar', in 24°S and 68°E, experienced a heavy gale, with thunder and lightening; ship hove on her beam ends; cut away top gallant mast; every plate, all clothing, and every book on board washed away. Barometer 29.30 inches."

While Meldrum was putting together figures for the Indian Ocean, Pogson, then Government Astronomer in Madras, was examining weather data for South-east India. His analysis led him to conclude that there was a correlation in that area, too, between variations in the rainfall and the sunspot cycle.

This concentration of effort on the weather of the Indian subcontinent and its environs was not dictated solely by the apparent simplicity of the weather cycle there. The problem of famine was then particularly evident in India—one series of famines in the 1860s being followed by another, even more acute, in the 1870s. As is evident from the meetings of the Indian Famine Commission at the end of the latter decade, the possibility of forecasting future famines

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Table 1 Rainfall (Paris) and sunspot cycle for the years 1824–1867

Number of year in mean cycle	Rainfall variation (inch)	Spot variation (number)
1	−2.0	−38.2
2	−0.9	−22.7
3	+0.8	−5.7
4	+1.9	+33.3
5	+1.9	+41.9
6	+1.8	+30.7
7	+1.1	+13.1
8	+0.2	−1.5
9	−0.5	−12.1
10	−0.8	−21.7
11	−2.0	−28.0

was under urgent discussion, and the discovery of correlations with the solar cycle seemed to offer the best hope of achieving this.

Studies of weather variation in other parts of the world were being conducted simultaneously, often by the same investigators. Typically, these consisted of an examination either of an extended time sequence of observations made at a single station, or of a shorter time sequence averaged over several stations. Thus Meldrum correlated sunspot numbers with rainfall data measured at the Paris Observatory. As the Observatory had been founded in the seventeenth century, it could provide almost two hundred years of observational material. (The sunspot cycle also had to be extrapolated over this period, of course, and Meldrum, like virtually all his contemporaries, made use of an extensive study of solar records carried out by Wolf at Zurich and published in the 1870s.) Meldrum then studied four spot cycles in more detail, using a shorter time base but averaging over more than 150 observing stations dotted all round the world (see Table 1). In both instances, he found an apparent correlation between the phase of the solar cycle and the weather record.

Rainfall was by no means the only parameter used to characterise terrestrial weather. Balfour Stewart used, in addition, atmospheric temperatures and the heights of rivers; whereas W. S. Jevons returned to Herschel's original approach, and linked the number of spots with the business cycle. But the variable that seemed to offer the best chance of providing a physical interpretation of the Sun–Earth interaction was the atmospheric pressure. Blanford⁴—yet another meteorologist working in India—developed the concept of a “barometric seesaw”; by which he meant an oscillation in pressure between two parts of the globe, that varied with the period of the solar cycle.

“Between Russia and Western Siberia on the one hand, and the Indo–Malayan region (perhaps including the Chinese region) on the other, there is a reciprocating and cyclical oscillation of atmospheric pressure; such that the pressure is at a maximum in Western Siberia and Russia about the epoch of maximum sunspots, and in the Indo–Malayan area at that of minimum sunspots.”

He interpreted this in terms of varying energy supply from the Sun, which led to an alteration in the amount of water evaporating into the atmosphere. This produced, in turn, fluctuations in pressure, temperature and rainfall.

In view of the numerous correlations between the solar cycle and the weather that were discovered in the last quarter of the nineteenth century, it seems at first sight surprising that the century ended with the matter still a focus for controversy. Three reasons can be discerned for the continued doubt.

These correlations represented the result of averaging over widely varying sets of values: their credibility therefore remained dubious in many eyes. As Blanford, who believed in the correlations, remarked, “The amount of the variation is so small in comparison with the irregular

vicissitudes of the weather, that it is for the most part obscured and unrecognisable, except fitfully, or in some few favoured regions.”⁵ The physical mechanism by which the solar–terrestrial interaction operated still remained unclear in spite of numerous speculations. Moreover, the observations seemed to support contradictory conclusions: the measurements of air temperature suggested greatest solar luminosity at sunspot minimum, whereas the study of rainfall and cyclones suggested greatest luminosity at maximum. It was expected that the discovery of correlations would lead on to the prediction, at least in general terms, of future changes in the weather. Yet even the intensive study of the Indian monsoons failed to lead to an acceptable forecast of famine conditions.

The main sticking point was the uncertainty regarding the exact nature of the supposed solar luminosity variations. Not only were the observations contradictory: so were opinions regarding the nature of the variations. The original idea had been that spots blanketed a part of the solar surface, thus reducing the energy emitted, and producing minimum flux at sunspot maximum. By the early twentieth century, it was, on the contrary, even being contended that the luminosity variations might rather be the result of changing numbers of solar prominences, so that maximum luminosity emitted might actually coincide with maximum spot number⁶. The obvious way of resolving this uncertainty was to make a series of direct measurements of the energy received by the Earth during a solar cycle. Throughout the nineteenth century, however, techniques were acknowledged to be insufficiently accurate for this kind of monitoring. There were, in fact, two obstacles in the way of measuring the solar constant. The first was the obvious need to develop a sufficiently sensitive bolometer. But the measurements had to be made at the bottom of the Earth's atmosphere; so, secondly, the effects of fluctuations in atmospheric transmission had to be eliminated. This effectively ruled out the British Isles as a site for the appropriate observations. In fact, interest in possible relationships between solar activity and the weather was clearly on the wane in this country just before World War I, and the renewed attack on the solar luminosity problem took place instead in the USA.

The Smithsonian Astrophysical Observatory in Washington had been involved since its foundation in 1890 in measuring the solar constant. Under Langley, the Observatory's first director, the main concern had been to develop as sensitive and accurate an instrument for this purpose as possible. But C. G. Abbot, the second director, decided not long before World War I that sufficiently good results were being obtained for small changes in the solar constant to be detectable. During the 1920s and 1930s he pushed ahead with the measurements, finding, so he believed, definite evidence for a variation in the solar luminosity from year to year which could be correlated with weather conditions on Earth.

Yet, in spite of this massive effort, *Nature* commented without fear of contradiction at the beginning of World War II that: “Many attempts have been made in the past to correlate solar and terrestrial phenomena . . . There is no direct meteorological effect of any serious importance.”⁷

Partly, no doubt, this could be explained by British insularity; for studies of Sun–weather relationships had been at a low ebb in this country since before World War I. But the Smithsonian results were also received with scepticism by many in the USA. The doubts were once more engendered by the difficulty of unravelling a small signal from a large amount of noise: most of Abbot's peers felt that, although his conclusions might ultimately prove to be right, they had not been definitely established. The apparent variations in solar energy might actually be caused by systematic errors, for example in correcting for the influence of the Earth's atmosphere.

Although Abbot continued to stress the significance of the Smithsonian results, the general feeling among both British and American scientists in the years following World War II seems to have hardened towards the view that the total energy emitted by the Sun did not vary appreciably during the solar cycle. This belief, which has strengthened with time⁸, obviously rules out the kind of interaction mechanism that was being considered before World War I. It is hardly surprising that interest in the whole subject of solar activity and the weather continued to flag. (Although discussions on the topic continued in the Soviet Union, they were relatively little noticed in the West.) The return of interest only followed, belatedly, after the growth of solar system studies in the 1960s.

The actual correlations studied in the last five years, or so, have not differed very greatly in principle from those we have noted before, although the amount of data that can be collected and reduced has now become a good deal larger. Thus one recent attempt to find correlations made use of a study of some ten million different measurements of air temperature⁹. But the crucial point has remained the question of the mechanism involved. However many numbers are invoked, the old doubts are unlikely to be resolved so long as all that can be offered is statistical regularities. If the Sun's luminosity can no longer be regarded as a variable, attention must naturally turn to those features of the Sun that might affect the Earth and that are certainly known to vary during the solar cycle—magnetic fields, particle emission and high energy radiation.

It has long been realised that changes in the upper atmosphere of the Earth can be related to the solar cycle. Balfour Stewart—one of the first to realise that the Earth's upper atmosphere is electrically conducting—attempted to correlate variations in terrestrial temperature and magnetic declination. The interactions here are between the Sun and the ionised upper atmosphere. In the 1960s, it became apparent that the neutral upper atmosphere was also strongly influenced by solar activity, and the belief grew that there might be further interaction with the lower atmosphere. The difference in the masses of the upper and lower atmosphere, however, suggested that such a link could not be direct; rather it would have to occur by some kind of trigger action, so that a small effect in the upper

atmosphere could produce a major effect in the lower atmosphere by way of an instability. An intriguing recent claim—that solar magnetic sector structure can influence terrestrial atmospheric vorticity¹⁰—may indicate the sort of direction in which this instability should be sought. Unfortunately, there is no scarcity of competitors.

As I have remarked, one thing a satisfactory Sun-weather relationship should be able to do is to forecast future changes in the weather. This obviously requires an ability to predict future solar activity; so it must surely rank as more than a coincidence that interest in such powers of prediction accompanied the upsurge of activity in correlating solar and terrestrial changes both in the nineteenth century and today. Even more interestingly, attempts have been made on both occasions to relate solar activity—measured by sunspot numbers—with particular alignments of the planets^{11,12}. Although the exact mechanisms have differed, the significance of such correlations is that, if true, they should permit the forecasting of solar activity for any time in the future, as planetary orbits are very well determined. It is a commentary on the overall problem of establishing anything firmly in this field that, both in the nineteenth century and now, there have been strong counterclaims that the apparent coincidences between planetary positions and sunspot numbers are simply figments of the analysis¹³.

Would it then be fair to conclude with Solomon that there is no new thing under the Sun? Not entirely—for, although in one sense additional correlations may add nothing new, they may lead to an acceptable mechanism for the necessary interaction between the Sun and the lower atmosphere of the Earth. If this could only be defined, the whole controversial field might be rapidly translated into the realms of high respectability.

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articles

Packaging of prophage and host DNA by coliphage λ

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Bacteriophage λ can package headfuls of prophage and host DNA. The resulting particles are not infective without removal in vitro of unpackaged DNA that protrudes from the head and prevents the attachment of tails. After tail attachment the particles can transduce a large number of host genes.

THE DNA of the phage λ virion (mature DNA) is a double-stranded molecule about 47,000 base pairs long with specific single-stranded sequences, 12 bases long, at each 5'-phosphate end^{1,2} (Fig. 1a). The left end sequence is complementary to the right end sequence; this enables circularisation of a molecule by base-pairing of the ends³. Mature DNA is formed by the cutting to size of a longer precursor, a linear polymer of mature molecules joined together head-to-tail

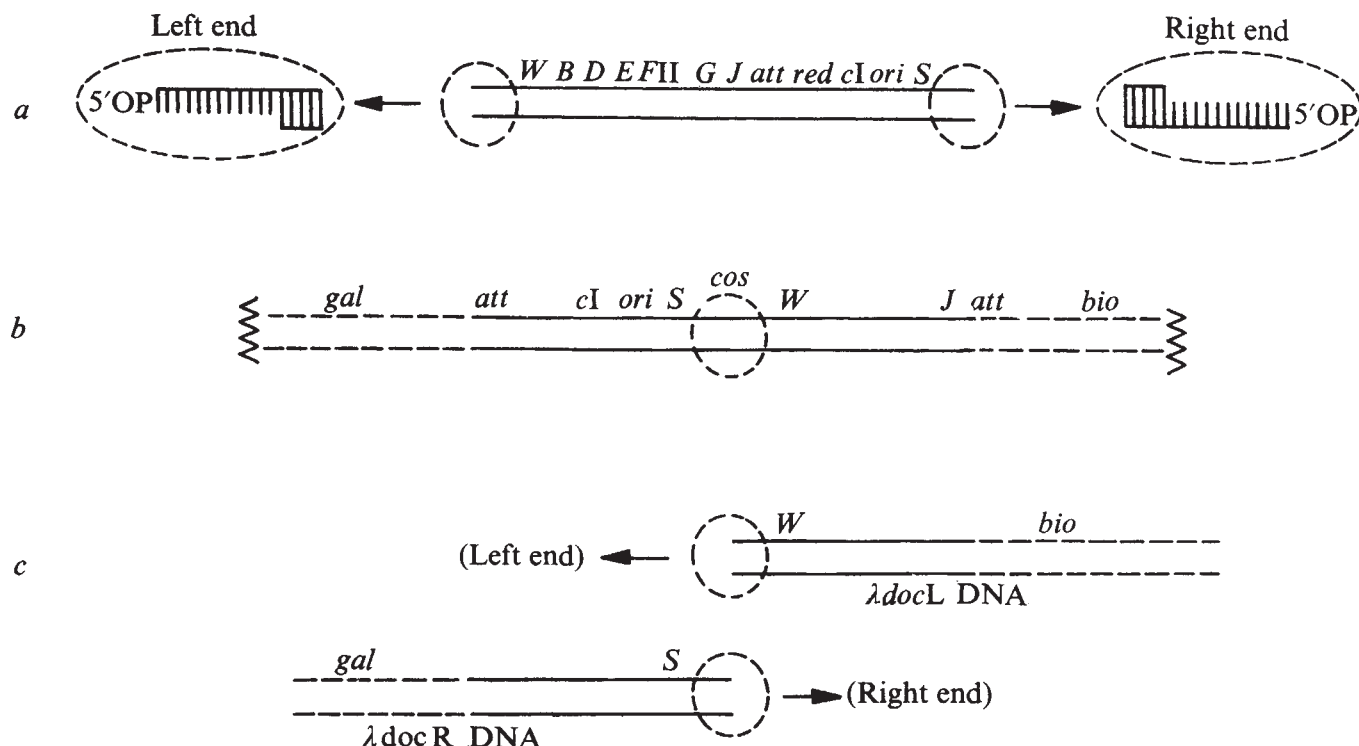


Fig. 1 *a*, Each strand of the DNA of the λ virion is represented by a straight line. The symbols above the double line give the order on the phage genetic map of λ genes mentioned in the text¹⁶. The distances between genes are arbitrary. The left and right ends of the DNA (within the circles) are shown in expanded scale to the left and right with the vertical bars representing bonds between base pairs, *b*, The inserted prophage and adjoining bacterial DNA are represented according to the same convention except that the bacterial DNA strands are shown by broken lines. Insertion occurs by reciprocal recombination between the site denoted *att* in *a* and another *att* in the bacterial DNA (ref. 26). During insertion the left and right ends of mature DNA are joined by base pairing and ligation^{6,26,31}. Consequently the prophage map is a cyclic permutation of the phage map, with the joined ends (within the circle under *cos*) approximately in the centre. *gal* and *bio* are bacterial operons closely linked to the prophage³³. *c*, Chromosomes of λ docL and λ docR are represented according to the same convention. The cutting and packaging of these DNAs are described in the text.

by covalent bonds^{4,5}. This is thought to occur as follows⁶⁻⁹. The products of λ genes *A* and *D*, in conjunction with a phage head precursor, make two staggered, single-stranded endonucleolytic cuts 12 base pairs apart at the regions of the polymer corresponding to the mature ends. The sequence-specific cutting activity is called Ter, for terminase, and its site of action is called *cos* (for cohesive end site). The DNA between an adjacent pair of cuts is condensed by an unknown mechanism to approximately 15 times its previous concentration¹⁰ and packaged into a phage head. Cutting and packaging have a common requirement for several phage and at least one host gene^{11,12}. Thus, the two reactions are coupled.

DNA cutting by λ differs from that by phage T4 (ref. 13) and phage P22 (ref. 14). Although both of these phages, like λ , cut and package DNA from continuous linear polymers, the length of the mature DNA is determined not by the distance between two specific cutting sites but by the capacity of the head; that is, headfuls of DNA, each slightly larger than the genome, are cut from the polymer. T4 DNA cutting seems to involve no unique recognition site on the DNA. In contrast, mature P22 DNA is formed by sequential cutting and packaging of headfuls from a unique starting site on a polymer¹⁵. The latter phage, therefore, combines some of the elements of both site-specific and headful cutting.

We report here the production of variant λ particles whose DNA, like that of T4 and P22, is packaged by the headful. These particles are the major product of phage growth when normal packaging is suppressed by the elimination of polymeric λ DNA. In such lysates we also find transducing activity for a large number of bacterial genes. The particles responsible for such activity seem to

be formed by the packaging of headfuls of bacterial DNA in λ capsids.

Packaging of prophage DNA

To examine packaging in the absence of polymeric λ DNA, we characterised the phage particles released after induction of an excision-defective monolysogen. In such a lysogen, phage DNA replication initiates in the prophage at *ori* (Fig. 1*b*) and then proceeds in both directions in the bacterial chromosome^{16,17}. The structure formed after several rounds of DNA synthesis have initiated is not known with certainty; it probably resembles a puff of localised replication around the prophage. If so, the prophage replicas would be colinear with an unreplicated prophage (Fig. 1*b*).

How is such DNA packaged? We propose the following model. Ter cuts the prophage at *cos* to form the normal left and right ends of mature λ DNA. DNA to the right of *cos* is preferentially condensed and packaged in phage heads. The amount of DNA per head equals the capacity of the head (a headful). Unpackaged DNA, continuous with the packaged DNA, protrudes from the head, blocking the attachment of tails and preventing the function of λ protein(s) that act at the head-tail junction.

Some evidence for this model already exists. Gottesman and Yarmolinsky¹⁸ and Little and Gottesman¹⁹ showed that Ter cuts *cos* after induction of an excision-defective lysogen. They also characterised the DNAs of two types of defective phage particles produced: λ docL and λ docR. λ docL DNA carries the left end and λ docR DNA the right end of mature λ DNA; both DNAs are colinear with the prophage and in addition carry adjacent bacterial genes (Fig. 1*c*). We have further characterised λ docL particles and measured

Table 1 Plaque formers and *bio* transducers produced after induction of an excision-defective monolysogen (strain NS490)

Activity	Units per induced cell		+DNase -DNase
	+DNase	-DNase	
<i>Bio</i> transduction	2×10^{-2}	3.3×10^{-4}	60
Plaque formation	3.8×10^{-5}	3.6×10^{-5}	1.0

The relevant genotype of NS490 is: *supOrecA*(λ b2red3cIts857 *Sam7*). The excision defect of NS490 is a consequence of inactivation of the right prophage attachment site by the *b2* mutation¹⁸. A lysate was prepared by heat induction of NS490 as follows. Cells were diluted from a fresh overnight culture into tryptone broth (1% Difco tryptone, 0.5% NaCl in distilled water) and grown in a shaking water bath with vigorous aeration at 32 °C to $A_{630} = 0.4$ (this corresponds to 2×10^8 viable cells ml⁻¹). At this point the temperature of the culture was raised rapidly to 42 °C for 15 min and then lowered to 38 °C. After 135 min of incubation at 38 °C the cells were collected by centrifugation, resuspended in 0.02 volume TMG (0.01 M Tris-HCl, pH 7.4, 0.01 M MgSO₄, and 0.01% gelatin) with or without the addition of pancreatic DNase (10 µg ml⁻¹) (Worthington, RNase-free), and incubated with gentle shaking for 30 min at 32 °C together with CHCl₃ (about 0.05 ml ml⁻¹) to promote lysis. The biological activity of such lysates was stable at 4 °C. *Bio* transducers were assayed by infecting a starved overnight culture carrying the *bioA24* mutation⁴⁹ with appropriate dilutions of the above lysate together with five λ cIts857 phage per cell. After 15 min incubation at 32 °C for phage adsorption, a constant number of cells (approximately 10^8) was added to 2.5 ml molten 0.7% agar in distilled water, and poured into Petri plates containing about 40 ml M9 agar⁵⁰ supplemented with 0.5% glucose, 0.5% Difco vitamin-free casamino acids, and thiamine (0.1 µg ml⁻¹). Colonies of *bio*⁺ transductants were counted after 24 h incubation at 32 °C. Plaque formers were assayed on strain YMC (*supF*⁺). Plaque formation and the growth of bacterial cultures for transduction were as described⁵⁰.

the number produced per cell relative to λ docR. The results of these studies are presented next.

Packaging of DNA to the right of *cos*

Our model predicts that an excision-defective lysogen will produce tailless phage heads carrying λ docL DNA. In fact, λ docL particles, as originally characterised, were uninfected, seemingly because they lacked tails¹⁹. If tail attachment to λ docL is prevented by uncut DNA protruding from

Table 2 Requirements for extra cellular maturation of λ docL particles

Limiting gene products	Relative infectivity of:	
	λ docL	λ docR
None	1.0	1.0
J	0.02	1.0
W	0.95	1.0
FII	0.02	1.0

A culture of NS490 was induced and collected as described in Table 1 and then mixed with an induced culture of a second lysogen (see below) in TMG to give about 10^8 and 10^{10} cells per ml, respectively, of the two strains. After 10 min incubation at 32 °C in the presence of CHCl₃ (0.05 ml ml⁻¹), DNase (10 µg ml⁻¹) was added and incubation continued for 30 min. The mixtures were then assayed for transducing particles. The numbers in column 2 (λ docL) are relative numbers of *bio* transductants (see Table 1). The value 1.0 corresponds to 0.01 *bio* transductants per input NS490 cell. When DNase was omitted from the mixture, this value fell to 0.0003 *bio* transductant per NS490. The numbers in column 3 (λ docR) are relative numbers of *gal* transductants. A value of 1.0 corresponds to 0.002 *gal* transductants per input NS490. *gal* transduction was measured as described in ref. 50 with strain SA624 (*galT*Am28)⁵¹ as recipient. The second lysogen was always a derivative of strain 594 (*supO* *srrR* *galK2* *galT1*). It was induced and collected as described in the legend to Table 1 for strain NS490. The prophage varied: in line (1), (None), it was λ Eam4 *cIts857* *Sam7*; in line (2), (J), λ Eam4 *Jam27* *cIts857* *Sam7*; in line (3), (W), λ Wam403 *Eam1100* *cIts857* *Sam7*; and in line (4), (FII), λ Eam1101 *FIIam423* *cIts857* *Sam7*. The purpose of the mutations in gene *E*, which encodes the major λ head protein, was to prevent the trapping of other phage proteins in or on head structures made of *E* protein, and to prevent the formation of *bio*-transducing particles by strain 594 lysogens.

the phage head, removal of the excess DNA should lift the block. We indeed observed a large increase in infectivity, measured by *bio* transduction, when we treated a λ docL lysate with DNase (Table 1, line 1). In contrast, DNase treatment did not increase the infectivity of plaque-forming λ in the same lysate (Table 1, line 2). The latter particles presumably have normal left and right DNA ends as both are required for autonomous phage growth^{20,21}. Therefore we do not expect that any DNA will protrude from the head and block tail addition.

If *bio*-transducing particles arise from λ docL heads, they will carry the left end of wild-type λ DNA and phage genes from the left arm of the λ chromosome. These predictions are confirmed by the following observations (the data will be presented elsewhere). The *bio*-transducing activity can be concentrated and purified by standard techniques (see Table 4). DNA extracted from purified particles carried the left but not the right end of wild-type λ DNA (ref. 22 and K. Murray, personal communication). The presence of the left end is consistent with the observation that this DNA possessed high transforming activity for *bio* in the transformation assay described by Kaiser and Hogness²³; such activity requires that the DNA carry at least one λ end²⁴. This DNA has been restricted by treatment with endonuclease *R.EcoRI*, and the resulting fragments resolved by electrophoresis in agarose gels. One of these fragments was indistinguishable in size from that of a 0.44 fractional length fragment cleaved from the left arm of λ DNA by the same enzyme (ref. 25 and D. Tiemeier, personal communication). If the DNA of the *bio*-transducing particles carries only one of the λ ends, it will be unable to circularise and therefore to form a prophage²⁶. In fact,

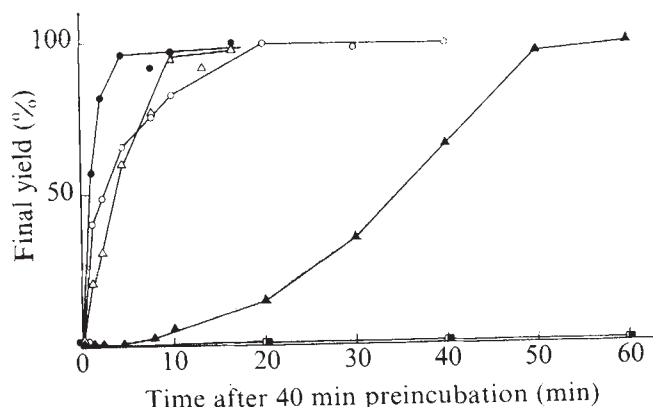


Fig. 2 The kinetics of the *in vitro* completion of λ docL virions. λ doc particles were produced by induction of *supO* *recA* (λ Jam6 *b2* red3 *cIts857* *Sam7*) as described in Table 1, without the addition of DNase. λ tails were produced in the same way by induction of strain 594 (λ Wam403 *Eam1100* *cIts857* *Sam7*), (see Table 2). Samples (0.2 ml) of the first lysate containing one drop CHCl₃ were preincubated for 40 min at 32 °C in the presence (+DNase) or absence (-DNase) of DNase (0.2 µg ml⁻¹). After this preincubation period (0 min on the figure) the following additions were made to the samples and aliquots were removed at the designated times to assay λ docL (*bio* transduction) or λ docR (*gal* transduction): (O), (+DNase)+0.2 ml tails, λ docL assayed; (■), (-DNase)+0.2 ml tails, λ docL assayed; (□), (+DNase)+0.2 ml TMG, λ docL assayed; (▲), (-DNase)+0.2 ml tails+DNase (0.2 µg ml⁻¹), λ docR assayed; (●), (-DNase)+0.2 ml tails+DNase (0.2 µg ml⁻¹), λ docR assayed; (△), (-DNase)+0.2 ml tails+DNase (10 µg ml⁻¹), λ docL assayed. The final yield of λ docL obtained in the presence of DNase and tails was 0.007–0.008 *bio* transductants per input *recA* cell. This is 70–80% of that obtained when the λ doc lysate, tails and DNase (10 µg ml⁻¹) were mixed immediately after lysis. Preincubation of the lysate for 40 min in the presence of tails followed by the addition of DNase (0.2 µg ml⁻¹) gave results identical to (▲). The final yield (0.0007 *gal* transductants per input *recA* cell) and kinetics of formation of λ docR were the same in the presence as in the absence of DNase.

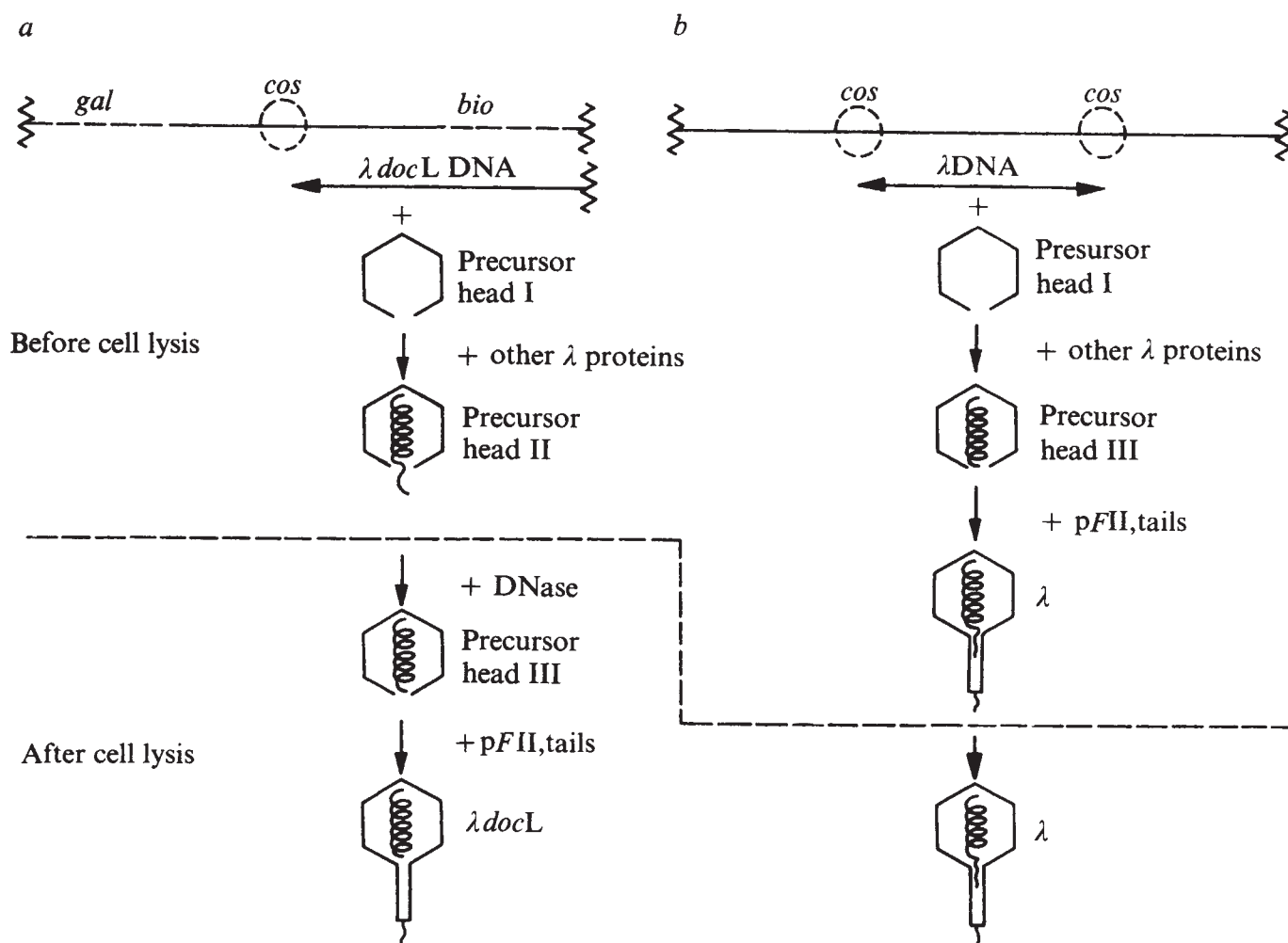


Fig. 3 Models for the packaging of prophage and polymeric DNA. *a*, First step in the packaging of prophage DNA is the cutting of *cos* and the encapsulation of DNA to its right by precursor head I together with the products of genes *A*, *D*, *F1* and *W*. The work of several groups shows that the formation of precursor head I (prehead or petit λ) requires the function of phage genes *B*, *C*, *nu3* and *E* (refs 8, 56 and 57), while the products of genes *A*, *D*, *F1* and *W* act subsequently^{7,8,29,40}. All of these genes are required for $\lambda docL$ production (data not shown). Packaged duplex DNA is shown as a coil; unpackaged duplex DNA as a straight or wavy line. In the lysate, the excess DNA protruding from precursor head II is trimmed by DNase action and the $\lambda docL$ particle is completed by the addition of *FII* protein and tails. *b*, Packaging of polymeric DNA differs from that of the prophage in that adjacent copies of *cos* are both cut, and the intervening DNA is packaged. Therefore, no excess DNA protrudes from the head, and *FII* protein and tails add intracellularly. We show DNA associated with the tail of λ but not with that of $\lambda docL$ (J. Thomas, N.S. and R.W., unpublished).

less than 1% of the *bio* transductants were lysogens. We have observed cotransduction of the *bioA* gene with λ left phage arm genes *B*, *D*, and *J* by using *bioA* recipients that carried suitably marked prophages. In the light of these observations, we refer here to the *bio*-transducing particles as $\lambda docL$ particles.

The hypothesis that functional tails add to $\lambda docL$ heads only after extracellular DNase treatment is confirmed by the following experiment. An induced excision-defective lysogen was diluted 100-fold with a second induced lysogen that carried a λJ^- prophage, and the mixture was lysed by adding chloroform followed by DNase. Only the excision-defective prophage can synthesise tails^{27,28}; thus, we predict that the formation of infective $\lambda docL$ should be limited by the low concentration of tails in this mixture. In fact, the number of *bio* transducers was 30-fold less than that found when the excision-defective lysogen was diluted with a tail-proficient lysogen (Table 2, lines 1 and 2). In contrast, the infectivity of phage particles whose DNA carried a wild-type λ right end ($\lambda docR$; Fig. 1c.) was unaffected by dilution with a tail-defective lysogen (Table 2). These observations show that the attachment of functional tails to $\lambda docL$ heads occurs extracellularly, whereas attachment to

$\lambda docR$ heads occurs intracellularly. Is this also true of λ proteins that act at the head-tail junction? To answer this question we diluted the $\lambda docL$ -producing lysogen before lysis with a *W*-defective or an *FII*-defective lysogen. The protein products of the *W* and *FII* genes are known to act sequentially on DNA-filled heads to permit the attachment of tails²⁹. After lysis of the mixtures, we found normal levels of *bio*-transducing activity in the *W*-deficient but not in the *FII*-deficient mixture (Table 2, lines 3 and 4). We conclude that *W* but not *FII* product can act on $\lambda docL$ heads intracellularly.

The above conclusions are consistent with the effect of varying the order of addition of tails and DNase on the subsequent rate of formation of infective $\lambda docL$. Figure 2 shows that if tails were added to a tailless $\lambda docL$ lysate before or at the same time as DNase, there was a delay in the formation of infective $\lambda docL$ relative to $\lambda docR$. The delay was eliminated when DNase was added to the lysate before tails. We conclude that DNA must be digested before functional tails can add to $\lambda docL$ heads. We suspect this is also true for the action of *FII* product.

Our model of the formation of $\lambda docL$ is compared with the formation of λ in Fig. 3.

Capacity of head and asymmetry of packaging

The amount of DNA per $\lambda docL$ particle that remains after DNase treatment should correspond to the capacity of the λ head (Fig. 3). This prediction can be verified by equilibrium density gradient centrifugation of a DNase-treated $\lambda docL$ lysate, as the density of a λ particle (and presumably of a $\lambda docL$ particle) is a known function of its DNA content¹. The results of such an experiment (Fig. 4) show that the density profile of *bio*-transducing activity had a peak corresponding to a DNA content 104% that of wild-type λ and a width suggesting some heterogeneity in the amount of DNA per particle. This is about the maximum amount of DNA that can be packaged in a λ head without a reduction of phage growth: a phage mutant with a DNA content of 109% had a burst size only 10% that of wild-type λ , and this reduction is directly attributable to the increased DNA size³⁰. A mutant with a DNA content of 106.5% ($\lambda imm434att^2$) had a larger but still reduced burst (N.S. and R.W., unpublished).

In addition to $\lambda docL$, an excision-defective lysogen produces another particle, $\lambda docR$, whose DNA consists of the right arm of the λ chromosome and bacterial DNA to the left of *attL*. It is formed by the packaging of prophage and bacterial DNA to the left of *cos*¹⁹ (Fig. 1c). $\lambda docR$ can be physically separated from $\lambda docL$ by equilibrium density gradient centrifugation (see ref. 19 for the density distribution of $\lambda docR$). We found that the number of $\lambda docL$ particles in a lysate exceeded that of $\lambda docR$ by a factor of 200–600 (Table 3, column 2). The great excess of $\lambda docL$ relative to $\lambda docR$ confirms our hypothesis that DNA to the right of *cos* is packaged preferentially. Data to be presented elsewhere suggest that this asymmetry is a consequence of asymmetry in *cos* (N.S. and R.W., unpublished).

How is $\lambda docR$ DNA cut from the bacterial chromosome? At present, we can only offer a tentative answer to this question. The right end of $\lambda docR$ DNA must be formed by *Ter* acting specifically at *cos*; the left end may be formed through nonspecific cutting by *Ter* or by some other intracellular nuclease. Little and Gottesman¹⁹ have shown that the left end is not at a specific site. The DNase independence of $\lambda docR$ infectivity (data not shown) suggests that the left end is formed intracellularly.

While measuring the number of particles in λdoc lysates, we found that the ratio of infective to total particles (the specific infectivity) of $\lambda docR$ was 100–200 times greater than that of $\lambda docL$ (Table 3, column 4). This is at least partly explained by the defective DNA injection of $\lambda docL$ particles (J. Thomas, N.S. and R.W., unpublished). We shall consider this point further below.

Generalised transducing particles

Lysates of an induced excision-defective lysogen also contained particles that transduced many different bacterial genes (Table 4). A detailed analysis of these particles will be presented elsewhere (N.S. and R.W., unpublished); we list here some of their salient properties. Transduction was abolished if the lysate was treated with anti- λ serum or if the bacterial recipient was unable to adsorb λ . Therefore, the activity is associated with λ virions. Generalised transducers were found in lysates of *recA* bacteria infected with $\lambda int^- red^-$. Therefore, neither prophage insertion³¹ nor any of the major recombination pathways of phage or host³² is required. In fact, the production of generalised transducing particles was reduced by the action of the Red recombination pathway. This observation is not understood. All the transductants examined were haploid and carried no phage markers. This suggests that transduction occurs exclusively by substitution of infecting DNA for homologous recipient DNA. Transduction by substitution is expected if the transducing DNA is unable to circularise²⁶. The transducing activity for markers close to *attL* was

lower in lysates made by infection than in those made by induction. We attribute this to preferential replication of bacterial genes near the prophage. The transducing activity of a lysate made by infection varied over a 100-fold range for different markers. This suggests that certain regions of the bacterial chromosome are preferentially packaged by λ . Certain pairs of closely linked bacterial markers—such as *argH-rif* and *gal-bio* (ref. 33)—were cotransduced by λ . The appearance of generalised transducing activity, like $\lambda docL$ infectivity, required DNase treatment of the lysate (Table 4) in the presence of FII protein and tails (data not shown). The density distribution of transducing activity for five of six markers examined coincided precisely with that of $\lambda docL$ (see Fig. 4; *his* was the exception).

How are generalised transducing particles formed? The following hypothesis, although difficult to test, is appealing because of its simplicity. We assume the existence of sites in the bacterial chromosome whose nucleotide sequence resembles that of *cos*. These sites are cut with low efficiency

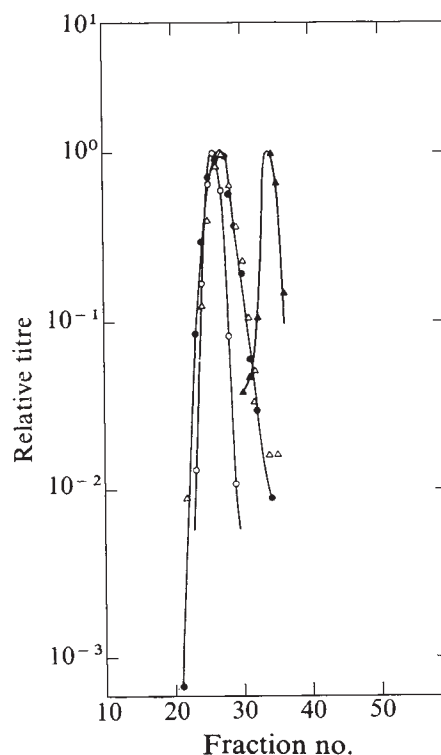


Fig. 4 A CsCl equilibrium density gradient of $\lambda docL$. A DNase-treated lysate of strain NS490, prepared as described in Table 1, was partially purified by low speed centrifugation (6,000g for 10 min at 4 °C) followed by centrifugation of the supernatant on a CsCl step gradient for 60 min at 4 °C in a Beckman SW56 rotor. (Four steps of densities 1.7, 1.55, 1.45 and 1.30 g ml⁻³ were used³⁸.) Fractions were collected from the bottom of the tube and those containing most of $\lambda docR$ and $\lambda docL$ infectivity were pooled and dialysed against 0.1 M Tris-HCl, pH 7.4, 0.01 M MgSO₄; 5 μ l of this was mixed with 2.484 g solid CsCl and 3.03 ml TMG containing $\lambda att^2 int am29 imm434 clts1$ (ref. 59) and $\lambda b2 imm\lambda cl$ (ref. 18). The DNA content of the former phage is 106.5% (ref. 1 and M. Fiant, *et al.* unpublished) and the latter 88% (ref. 1) that of λ . These phages were centrifuged for 22 h at 35,000 r.p.m. in a Beckman SW50 rotor at 4 °C. Sixty-two fractions were collected from a hole punctured in the bottom of the tube and assayed for $\lambda docL$ (●) (*bio* transducers; Table 1); *trp* transducers (△), (using strain C224 *trp* (λ)⁶⁰ as host; Table 4); λatt^2 (○); and $\lambda b2$ (▲), (turbid and clear plaque formers, respectively, on the non-lysogenic strain C600, plates incubated at 32 °C). The $\lambda docR$ activity (Table 3) was found in a broad peak within fractions 30–37 (data not shown). Densities were related to DNA contents by the equation of Bellett *et al.*⁹¹. In a separate experiment, A_{260} of the fractions was measured as well. The absorbance profile coincided with that of $\lambda docL$, the predominant λdoc species (Table 3).

Table 3 Yield per cell of $\lambda docL$ and $\lambda docR$

Phage	Units per induced cell		Specific infectivity (b/a)
	(a) Total particles	(b) Infective particles	
$\lambda docL$	4	0.02	0.005
$\lambda docR$	0.01–0.02	0.01	0.5–1

A DNase-treated lysate of NS490 derived from 300 I induced cells was prepared as described in Table 1 except that the initial concentration factor was 1,000-fold rather than 50-fold. Bacterial debris was removed by low-speed centrifugation, and the phage were sedimented by overnight centrifugation at 6,000g at 4 °C. The pellet was resuspended in 30 ml TMG, and the phage purified by centrifugation first through a CsCl step gradient and then in a CsCl equilibrium gradient. The latter step separates $\lambda docL$ from $\lambda docR$ (see Fig. 4). $\lambda docR$ was assayed by plaque formation on strain N585 (ref. 19), [*sup0* ($\lambda Nam7 imm434; 21hy5$)]. This assay, which depends on recombination between the *immL* marker of $\lambda docR$ and the right arm of the prophage, is about two to fourfold more efficient than *gal* transduction (data not shown). $\lambda docL$ was assayed by *bio* transduction (see Table 1). To measure total particles, the CsCl equilibrium gradient fractions containing most of the $\lambda docL$ and $\lambda docR$ infectivity were pooled separately and dialysed against TMG. A_{260} of each pool was measured and the values obtained converted to numbers of physical particles by assuming that an A_{260} of 1.0 corresponds to 3×10^{11} plaque-forming units per ml wild-type λ (ref. 52), and that the specific infectivity of wild-type λ is 1.0. The number thus obtained for $\lambda docL$ was confirmed by comparing the serum blocking power^{33, 34} of the pooled $\lambda docL$ with that of $\lambda cI ts857 Sam7$ purified in the same way. (The titration of neutralising antiserum with λ virions presumably measures the concentration of tail fibres.) Identical results were obtained with a second 300 I preparation and, for $\lambda docL$, with 20 ml lysate labelled with 3H -thymine.

by Ter, and adjacent DNA to the right is packaged according to the model we have proposed for $\lambda docL$. At present we have insufficient evidence to distinguish this model from one that assumes nonspecific cutting either by Ter or by another nuclease. In addition, it remains to be shown that the transducing particles contain exclusively bacterial DNA. Nevertheless, the coincidence between the density distribution of most of the transducing activity and that of $\lambda docL$ strongly suggests that λ packages bacterial DNA by the headful in these conditions.

Discussion

How are the packaging of prophage and polymeric DNA related? Our models of the two processes (Fig. 3) differ in one important respect: precursor head II, the DNA of which is continuous with unpackaged DNA, is unique to prophage packaging. We know little about the properties of this intermediate and are attempting to confirm its existence by electron microscopic examination of purified preparations. Because of the higher concentration and consequent lower entropy of packaged DNA, we might expect precursor head II to be unstable. On the contrary, it seems to be stable for at least 40 min in a concentrated lysate (Fig. 2). (This conclusion rests on the assumption that a constant fraction of the precursor head II present in concentrated lysate is converted to *bio*-transducing particles by treatment with DNase.) In contrast, similar structures formed during DNA packaging by phages T4 and T7 (prohead III (ref. 34) and the 40S structure³⁵, respectively) seem to be unstable. This difference may reflect a difference in experimental conditions or a difference in the precursors.

Our results indicate that the prophage DNA to the right of *cos* is packaged more efficiently than DNA to the left. The results of Emmons³⁶ demonstrated an asymmetry in the packaging of polymeric DNA. He explained this by proposing that packaging initiates at *cos* and proceeds rightwards. This proposal is completely consistent with our findings.

We find that the yield of $\lambda docL$ particles from an induced excision-defective single lysogen is four per cell (Table 3). This suggests that *cos* is cut at least once in every cell.

Freifelder *et al.*³⁷ have proposed that two copies of *cos* on a single molecule are required for optimum Ter cutting. Our measurements of the total yield of $\lambda docL$ do not support this proposal: the yield is no greater in a tandem polylysogen than in a single lysogen even though the production of plaque-forming particles is much higher in the former strain (N.S. and R.W., unpublished). We are measuring the kinetics of $\lambda docL$ production in the two strains.

The failure of tails to add to precursor head II is not surprising in view of the intimate and specific association between the tail of the wild-type virion and the right end of mature DNA (refs 38 and 39). Incubation of precursor head II with DNase in the presence of FII protein and tails markedly increases the infectivity of $\lambda docL$ (Table 1). Nevertheless, fewer than 1% of the total particles are infective (Table 3). The uninfected particles lack neither tails nor FII protein. The presence of tails and therefore of FII protein^{29, 40} is shown by: the concordance of the serum-blocking power with the A_{260} of purified $\lambda docL$ (see legend to Table 3); the concordance of the CsCl equilibrium density profile of the absorbance with that of the *bio*-transducing activity (see legend to Fig. 4); and electron microscopic observations of the particles adsorbed to bacteria (J. Thomas, N.S. and R.W., unpublished). Therefore, an explanation of the low specific infectivity of $\lambda docL$ must be sought elsewhere. We find that it results, at least in part, from inefficient DNA injection by tailed particles, and we postpone further discussion to a later paper.

Many bacteriophage species mediate generalised transduction. For some we know that the transducing particles contain little or no phage DNA (ref. 41, 42); apparently these phages can cut and package the bacterial DNA. We suspect that λ generalised transducing particles also contain exclusively bacterial DNA, although this has not been proved. If this DNA is packaged according to the model proposed earlier, only one Ter cut in the bacterial chromosome is needed for each particle. The absence of generalised transduction when DNase is omitted can then be explained by the improbability of finding in the bacterial chromosome two Ter-specific cutting sites that are separated by the length range compatible with phage viability (about 36,000–51,000 base pairs^{30, 43}).

How do other phages that normally contain DNA with specific ends package bacterial DNA in the absence of DNase? For phage P22 the answer may lie in the model of DNA packaging proposed by Tye *et al.*¹⁵, elaborated by Chelala and Margolin⁴⁴: only the first cut in the bacterial chromosome need be specific; other, nonspecific cuts can

Table 4 Production of generalised transducing particles

Marker transduced	Units per induced cell	
	DNase	DNase
<i>gltA, nadA, aroA</i>	10^{-3}	$< 10^{-5}$
<i>purE, pyrD</i>	10^{-4}	$< 10^{-6}$
<i>trpE, proA</i>	2×10^{-5}	$< 2 \times 10^{-7}$
<i>thr, leu, argH, pyrC</i>	$2-4 \times 10^{-6}$	$< 10^{-8}$
<i>his</i>	5×10^{-7}	$< 10^{-8}$

DNase-treated and untreated lysates of strain NS490, prepared as described in Table 1, were assayed for transducing activity on the following hosts: AB1157, obtained from the NIH collection, for *argH, proA, his, leu* and *thr*; X9170 and MP005, obtained from Dr W. Epstein, for *gltA* and *nadA*, respectively; AB478 and MSO, obtained from Dr S. Adhya, for *aroA* and *pyrD*, respectively; X5214, obtained from Dr E. Signer, for *pyrC*; X821, obtained from Dr R. Curtiss III, for *purE*; and *Y mel trp* E5947, obtained from Dr C. Yanofsky, for *trpE*. All strains not already lysogenic for λ were lysogenised to reduce killing by plaque-forming phage present in the lysate. Transduction was performed as described for *bio* (see Table 1) except that no co-infecting λ was added, and vitamin-free casamino acids were omitted from the plating agar for transduction of *gltA, nadA, purE, pyrD, proA, thr, leu, argH, pyrC* and *his*.

be made by a nuclease that is associated with the phage head. In the case of phage Mu-1 (ref. 45) one end of the phage DNA is known to be variable, both in nucleotide sequence and in length⁴⁶. It is therefore possible that a sequence-specific nuclease cuts one end of both phage and transducing particle DNA, while the other end is cut non-specifically. Generalised transduction by phage T1 (ref. 47) is more difficult to explain. Normal T1 DNA is linear with a duplex terminal repetition⁴⁸. The extent of the repetition varies slightly in extent at both ends of the DNA (ref. 48). This imprecision in the ends suggests that the T1 DNA cutting mechanism may be more tolerant than that of λ to sequence variability. Alternatively, the production of T1 generalised transducing particles may require a nuclease not normally used to cut the phage DNA.

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Two genes in the major histocompatibility complex control immune response

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Antibody production in mice is controlled by two functionally distinct gene types in the I region of the major histocompatibility complex. One controls T cell recognition of antigen and T cell mediators of cell cooperation, and the other controls the B cell response to T cell mediators.

THE immune response to various antigens is under the controlling influence of genes in the major histocompatibility complex (MHC), termed immune response or *Ir* genes¹⁻³. These genes are probably present in all vertebrates including man^{4,5}, where they may influence the outcome of various diseases^{5,6}. The responses controlled by these genes are dependent on thymus-derived lymphocytes or T cells, and include cell-mediated immune responses (for example delayed hypersensitivity) and thymus (T)-dependent antibody responses. A major feature of the MHC-linked *Ir* genes is that they show apparent antigen specificity, though they are clearly separate from the genes coding for serum antibody¹⁻³. We have been concerned mainly with the role of *Ir* genes in T-dependent antibody responses, especially in the cooperative interaction which is required between T cells and bone-marrow derived lymphocytes (B cells) for the latter to be triggered to antibody production.

In the past, attention has focused on whether the *Ir* genes code for the antigen-recognition system of T cells—that is the T cell antigen receptor¹⁻³, a structure which is known to exist but which has proved very hard to characterise. A problem with this interpretation of *Ir* gene function has been that in certain experiments—including our own—the locus of the action of *Ir* genes seems to be in B cells⁷⁻¹⁰. These contradictions can be reconciled if there are two kinds of *Ir* gene controlling the response to each antigen, one gene expressed in T cells and the other in B cells, with different functions in each cell type. We have developed assays for the products of the *Ir* genes and found strong evidence to support a two-gene model.

T cell factor

Our experiments have depended on the production by T cells of an antigen-specific molecule termed a T cell 'factor' which is capable of replacing T cells in thymus-dependent antibody responses¹¹. Figure 1 summarises the production and assay of this factor. We have shown that the factor carries a binding site for antigen by absorption with antigen-coated columns. Furthermore, the factor is not an immunoglobulin—it has a molecular weight of about 50,000, and does not react with anti-immunoglobulin reagents. On the other hand, the factor

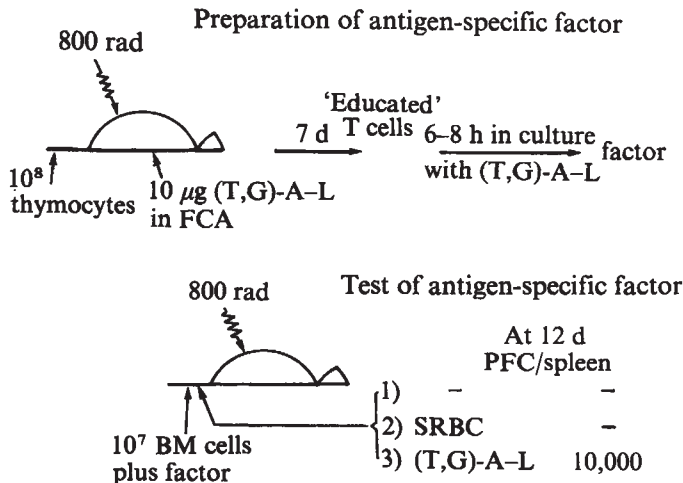


Fig. 1 Preparation and test of antigen-specific T-cell factor. The factor was prepared from mouse thymocytes which had been specifically primed or 'educated' to a synthetic polypeptide antigen such as (T,G)-A-L (poly (Tyr, Gln)-poly DL Ala-poly Lys)^{17,18}. The educated mouse T cells were cultured for 6-8 h with antigen *in vitro*, then removed by centrifugation. The culture supernatant was the source of T cell factor. Its activity was assayed *in vivo* by transfer together with bone marrow cells and antigen, (T,G)-A-L, into a lethally irradiated recipient syngeneic with the bone marrow donor. 12-14 d later the spleens of these animals were found to contain specific antibody producing cells against (T,G)-A-L which can be enumerated as plaque-forming cells (PFC). Factor produced against (T,G)-A-L will not stimulate a response against an unrelated antigen such as sheep red cells (SRBC)¹¹.

does react with antisera raised against the major histocompatibility complex of the mouse (*H-2*), and using region-specific antisera we have mapped the genes involved to the *I* region of *H-2*^{12,13}. These properties suggest that the T cell factor is the soluble expression of the T cell antigen receptor, and that the latter is coded for, at least in part, by the *I* region of *H-2*. Work from other laboratories supports this view. For example, a very similar antigen-specific molecule—though functionally suppressive—has been described by Tada and coworkers^{14,15}; in addition, other T cell factors, though lacking antigen specificity, are also products of the *I* region, and probably closely related to the specific T cell factors^{3,16}.

Detection of B cell acceptor

Since the *I* region contains the *Ir* genes, the T cell factor is an obvious candidate for a product of these genes. Ability to produce the factor could therefore be used as an assay for *Ir*-gene function, and it would be predicted that in at least

Table 1 Responses of high responder and low responder bone marrow cells to (T,G)-A-L and T cell factor in *F₁* recipients

T cell factor*	Bone marrow cells	Log ₁₀ PFC/spleen
No†	High responder (B10)	1.311
Yes	High responder (B10)	4.623
Yes	Low responder (B10.A)	1.462
Yes	<i>F₁</i> (B10 × B10.A)	4.724
	Standard error	0.184

*Factor specific for (T,G)-A-L was prepared in B10.BR (*H-2^k*) mice, low responders.

†Control: bone marrow cells transferred into irradiated recipients with antigen but without factor.

Responses given by 10⁷ bone marrow cells when combined with 10 µg (T,G)-A-L and the (T,G)-A-L specific T cell factor, and transferred into irradiated *F₁* (B10 × B10.A) recipients. The bone marrow cells were of either high responder (B10), low responder (B10.A) or *F₁* origin. Direct anti-(T,G)-A-L PFC measured in spleens of recipients 14 d after transfer. Results as log₁₀ geometric means, standard error shown.

some cases, low responders would lack the ability to make the T-cell specific factor. When this was investigated using as antigen the synthetic polypeptide (T,G)-A-L^{17,18}, for which high and low responders are known it was found that a low responder strain (C3H/HeJ, *H-2^k*) produced the cooperative T cell factor as efficiently as high responders (C3H.SW, *H-2^b*). On the other hand, the factor, whether of high or of low responder origin, would only cooperate effectively with bone marrow cells of high responder origin¹⁰. From these experiments, it was concluded that the defect in low responders (of *H-2^k* haplotype) was expressed in the function of bone marrow cells rather than T cells. Table 1 shows an experiment to confirm this result. In this experiment, *F₁* irradiated animals (B10 × B10.A) were used as recipients for either high responder (B10 or *F₁*) or low responder (B10.A) bone marrow cells, which were transferred with the same T cell factor and antigen. It is seen that only the high responder bone marrow cells responded. This experiment also serves to rule out possible contributions of the irradiated recipients to the result, since identical *F₁* (responder) recipients were used throughout.

In summary, *Ir* genes can be expressed in bone marrow cells, where they might function to receive the T cell product. It has already been predicted that B cells carry an 'acceptor' site for T cell factors¹⁹, and it seems likely that this could be where the bone marrow defect is localised. A direct assay for

Table 2 Absorption of T cell factor by high and low responder bone marrow cells

T cell factor*	Bone marrow cells used for absorption†	Log ₁₀ PFC/spleen
No‡		1.261
Yes		4.230
Yes	High responder (B10)	1.799
Yes	Low responder (B10.A)	4.518
	Standard error	0.330

*Factor specific for (T,G)-A-L was prepared in B10.BR (*H-2^k*) mice.

†Factor sufficient for five recipients was absorbed with 5 × 10⁷ bone marrow cells.

‡Control: response in absence of factor.

T cell factor for (T,G)-A-L was absorbed with either high or low responder bone marrow cells, and any remaining factor was mixed with *F₁* (B10 × B10.A) bone marrow cells and (T,G)-A-L and transferred into irradiated *F₁* recipients. Direct PFC to (T,G)-A-L were measured 14 d later in the spleens of the recipients. Results as log₁₀ geometric means.

the presence of an acceptor site on bone marrow cells is measurement of the ability of these cells to absorb T cell factor. As shown in Table 2, high responder (B10) bone marrow cells completely absorb the factor activity; low responder (B10.A) bone marrow cells, in contrast, do not absorb the factor at all. Similar experiments showed that the origin of the factor (that is, high or low responder) did not affect the result and that factor could also be absorbed in the absence of antigen. These experiments provide strong evidence for the existence of an acceptor in high responder, but not in certain low responder, bone marrow cells. Since bone marrow contains diverse cell types we have begun experiments with purified cell populations. Our preliminary results show that the acceptor is present on peripheral B cells (anti-0-treated lymph node cells depleted of macrophages); the presence of an acceptor on macrophages and peripheral T cells has yet to be established.

It was clearly important for the interpretation of these experiments to show that the factor was taken up by B cells in a biologically meaningful way. This was demonstrated by transferring bone marrow cells which had adsorbed factor into lethally irradiated recipients with antigen but without further addition of factor. It was found that provided the adsorption

had occurred in the presence of the specific antigen, the bone marrow cells made a good response on transfer. Presumably the antigen was necessary to focus factor on to the antigen-specific B cells.

The absence of the acceptor site from the B cells of a low responder to (T,G)-A-L, indicated that *Ir* genes might directly code for the acceptor. Table 3 shows that functional binding of factor to the acceptor site can be blocked by anti-H-2 sera. Bone marrow cells of F_1 origin (high $\times$ low responder: B10 $\times$ B10.A) were treated with anti-H-2 sera before absorbing factor in the presence of antigen. Anti-H-2^b (that is, antibody to the high responder haplotype) prevented the bone marrow cells from functionally absorbing the factor, as measured by the response of the bone marrow cells in irradiated recipients, whereas anti-H-2^a (the low responder haplotype) did not block the acceptor. Thus the acceptor seems to be coded for by genes in the *H-2* complex, and experiments are in progress to determine whether it is an *I*-region product, as predicted.

In summary, we have identified two products of the *H-2* complex, both probably coded by the *I* region, which are involved in T-B cell cooperation, namely the T cell factor and the B cell acceptor. As described above, there exist strains which are low responders to (T,G)-A-L because they lack the B cell acceptor. Do all non-responders share a B cell defect, or do some strains have T cell deficiencies which take the form of failure to make the specific cooperative factor? We have screened several strains for their ability to make T cell specific factor and for their B cell acceptor function. Table 4 summarises the results, and shows that while several strains have only B cell defects, one strain (B10.M, *H-2*^d) has a 'pure' T cell defect, and another (SJL, *H-2*^s) lacks both T and B cell function³². These findings make it very likely that two *Ir* genes are involved in control of the antibody response to (T,G)-A-L, one expressed in T cells, the other expressed in B cells. In order to confirm the two-gene hypothesis, we have studied the response of F_1 s between non-responders of complementary type (that is, T or B cell deficient). As shown in Table 5, such F_1 s are high responders. This complementation shows that almost certainly two genes affect the antibody response to specific antigens. Since in one F_1 combination (B10.BR $\times$ B10.M) the mice were congenic resistant and differ only at *H-2*, both the genes are located in the *H-2* complex, and from what has gone before, probably both in the *I-A* subregion. Backcross experiments are in progress to confirm this.

Taken together, the results described here provide strong evidence that two functionally distinct genes in the MHC determine the level of T-dependent immune responses by their effect on cell interaction. Other workers have found evidence for two *Ir* genes in the MHC by analysis of F_1 hybrids²⁰⁻²².

Table 3 Blocking acceptor with anti-H-2 sera

Bone marrow cells	Factor*	Antiserum pretreatment of bone marrow cells†	Log ₁₀ PFC per spleen
F_1 (B10 $\times$ B10.A)			
	Yes	—	2.857
	Yes	Anti-H-2 ^b	1.397
	Yes	Anti-H-2 ^a	3.230
		Standard error	0.250

*Specific for (T,G)-A-L, prepared in B10.D2 (*H-2*^d) mice.

† 5×10^7 bone marrow cells in 1 ml medium were heated with 0.1 ml undiluted antiserum for 30 min at 4 °C and then washed. Factor and (T,G)-A-L were then added and incubated with the cells for a further 30 min. The cells were again washed and then transferred into five irradiated F_1 recipients. Antisera used were B10.D2 anti CWB (anti-H-2^b) and B10 anti A/J (anti-H-2^a).

T cell factor specific for (T,G)-A-L was absorbed, in the presence of (T,G)-A-L, on to either normal or antiserum-treated F_1 (B10 $\times$ B10.A) bone marrow cells. The cells were then transferred, without further addition of factor, into irradiated F_1 recipients. The direct anti-(T,G)-A-L PFC responses in the spleens of the recipients were measured 12 d later. Results as log₁₀ geometric means.

Table 4 Cellular defects in antibody response to (T,G)-A-L

H-2	Strain	Response	T cell factor	B cell response
b	B10, C3H.SW	High	Yes	Yes
d	B10.D2, BALB/c	High	Yes	Yes
k	B10.BR, C3H/HeJ	Low	Yes	No
q	DBA/1	Low	n.d.	No
j	I.St	Low	Yes	No
f	B10.M	Low	No	Yes
s	SJL	Low	No	No

Data from refs 10, 12, 32 and unpublished.

For example, Rüde and Günther found complementation in the response to (T,G)-A-L in rat F_1 hybrids²¹, and more recently Benacerraf and colleagues have shown that two *Ir* genes control the response to the synthetic polypeptide GLPhe¹¹ in mice²². In the latter case, the genes were shown to map in different *I* subregions. It seems reasonable to expect that at least two MHC-linked genes will be found to determine the response levels to each thymus-dependent antigen. Our experiments take these findings further by defining the mode of action of the genes and their products. Thus one type of *Ir* gene codes for the antigen-specific T cell regulators of the immune response, and by inference, the T cell antigen recognition system. The second type of gene, by coding for an acceptor for the T cell regulators on lymphocytes determines the ultimate response of the cell to antigen (Fig. 2).

Model accounting for antigen specificity

We must now consider how the two genes would incorporate the antigen specificity which is the remarkable feature of the genetic control of the immune response. In the case of T cell defects in response an obvious possibility is that failure to respond to an antigen is due to the absence of the specific binding site for that antigen from the repertoire of T cell antigen receptors. The presence of *Ir* genes expressed in the B cell, however, requires a more complex explanation for the apparent antigen specificity of *Ir* gene control.

A model to account for the findings is as follows: (1) We propose that there must be several 'classes' of T cell factor,

Fig. 2 Cell interaction in the antibody response. Model showing the possible relationship between T cell receptor, specific T cell factor and B cell acceptor in cell cooperation and a mechanism of B cell triggering. 'Ia' refers to the antigenic determinants carried by the T cell factor detected by antisera raised against the *I* region. The *Ir* genes are believed to code for the T cell receptor, the T cell factor and the B cell acceptor.

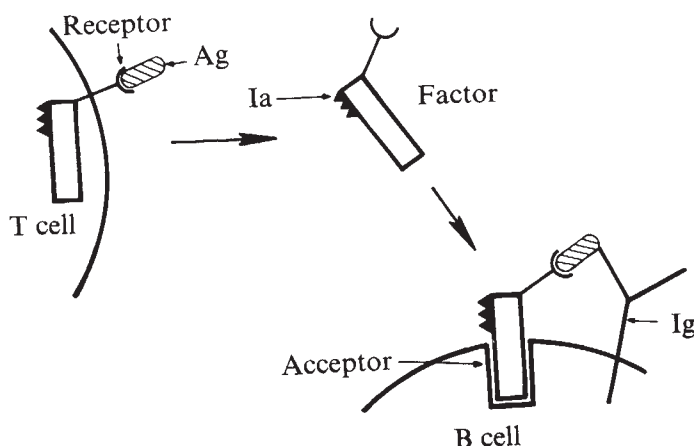


Table 5 Antibody response to (T,G)-A-L in F_1 hybrids of low responder strains

Strain	H-2	Response (PFC per spleen)
B10*	b	4.162
I.St	j	1.681
B10.BR	k	1.342
B10.M	f	1.079
(I.St $\times$ B10.M) F_1	j/f	3.875
(B10.Br $\times$ B10.M) F_1	k/f	4.291
	Standard error	0.362

*Control high responder.

Primary antibody responses, measured as direct anti-(T,G)-A-L PFC, 14 d after inoculation of $10 \mu\text{g}$ (T,G)-A-L in complete Freund's adjuvant, intraperitoneally. Log_{10} geometric means.

analogous in a sense to the classes of immunoglobulin and that for each factor class there exists a corresponding acceptor. The genes for a T cell factor class and the corresponding acceptor for that class comprise a 'set'. Note that the T factor is assumed to be clonally expressed, whereas each B cell would carry all classes of acceptor site. (2) Within each set there would be genes which contribute to the antigen-binding sites carried by that class of factor. Taking again the analogy with immunoglobulin genes, each set would contain a number of 'variable' (V) genes which could become associated with the 'constant' (C) gene for the T factor of that set (Fig. 3). (3) An important point is that the V genes of any one set will only code for part of the total binding site repertoire of T cell factors. In this way there will be antigens which will only react with the binding sites of one set, although there will be other antigens for which several sets are available. (4) The loss of a B cell acceptor for one class of T factor will render the B cell incapable of response to factor of that class, and thus to any antigen which is dependent solely on that set for cell cooperation. This would predict that all antigens which are restricted to that defective set would fail to give responses. (5) Two types of T cell defect are possible, namely (a) a defect in a specific antigen binding site, as already mentioned, and (b) a defect in the constant region of the T cell factor, leading to the effective inactivation of the entire set. (6) A consequence of the model is that 'Ir-gene' control will only be noticeable for antigens which are restricted to the binding sites of one set. This explains the well-known observation that genetic control is in general only observed for antigens where a limited number of determinants are presented to the

system, for example synthetic polypeptides, proteins in limiting dose, and alloantigens¹. (7) The different patterns of response which antigens show in different strains^{1-3,23}, arise in part because of the different genetic causes of low responsiveness. Furthermore, there is the possibility of genetic rearrangements, so that the genes comprising each set can vary between strains.

Although this model explains a great many of the phenomena associated with *Ir* genes, there are certain observations which require further discussion. For example, while we have shown that T cell factor can be produced to (T,G)-A-L in certain low responder strains, these same strains (notably $H-2^k$) lack other specific T cell-dependent activities such as delayed hypersensitivity (Davies and Shearer, personal communication), and proliferation of T cells in response to antigen *in vivo*²⁴, and *in vitro*²⁵. This dichotomy could be explained if T cell-T cell interaction were necessary for the generation of effector T cells, and if this interaction took place by the same molecular mechanisms as T cell-B cell cooperation. Alternatively, suppressor T cells may be generated in situations where low response results from an acceptor defect^{26,27}. A further problem arises from the response to (T,G)-A-L in tetraparental mice constructed from high responder ($H-2^b$) parents, where low responder B cells produced antibody to (T,G)-A-L²⁸. This is at first sight difficult to reconcile with our demonstration of an acceptor defect on $H-2^k$ B cells. But it may be that conditions in the responding tetraparental create an environment in which the acceptor defect can be bypassed. Alternatively strains nominally of the same $H-2$ haplotype may have undergone rearrangement or mutation of genes in the *I* region. The locus of unresponsiveness in the animals used to construct the tetraparentals would have to be determined to distinguish these possibilities.

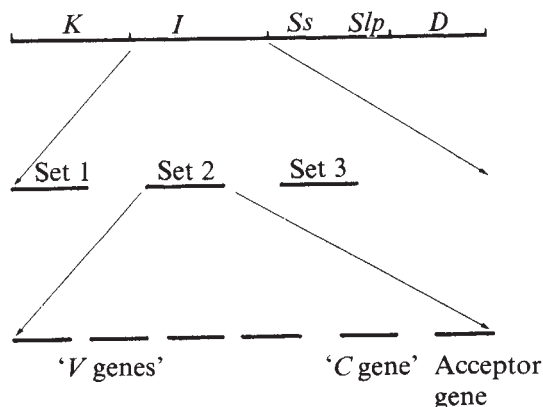
Finally, the emerging significance of the *I*-region products, not only in genetic control, cell cooperation and factor production, but also in mixed lymphocyte reaction²⁹, graft-versus-host response³⁰, immunological enhancement³¹, and so on, suggests that these phenomena may all be reflections of the same molecular structures.

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Fig. 3 Immune response genes of the $H-2$ complex. Schematic representation of the possible arrangement of genes in 'sets' in the *I* region of $H-2$. Within each set are genes coding for the binding site of the T cell receptor and factors 'V genes', for the 'constant' region of the receptor and factors 'C genes', and for the acceptor.



letters to nature

X-ray observations of Cyg X-1 with ANS

FROM May 1 to May 8, 1975, Cyg X-1 was observed with the instruments on board the Astronomical Netherlands Satellite (ANS). We report here the results of the measurements performed with soft and hard X-ray detectors of the Space Research Laboratory in Utrecht and the Center for Astrophysics in Cambridge, USA. Cyg X-1 underwent an upward transition in its intensity¹, that seems to be the inverse of the downward transition seen by Uhuru in April 1971. The bulk of the increase occurs at low energy: between 1 and 2 keV, the intensity increased by a factor of 10 over the November 1974 intensity observed by ANS, while above 8 keV there is no significant change.

The Utrecht experiment consists of two detectors. A 1.7 μm titanium window proportional counter of 45.0 cm^2 effective area collimated to a field of view of $36' \times 90'$ (FWHM) is sensitive in the energy range 0.35–0.45 keV and 1–7 keV, divided over 6 pulse height channels. The second instrument, a parabolic reflector with a projected area of 144 cm^2 , has in its focus a small-area counter with a window of 3.6 μm polypropylene. The field of view is a $34'$ circle. A full description of the instrument is given in ref. 2.

The relevant portion of the Cambridge experiment consists of 50 μm beryllium window proportional counters of 50 cm^2 effective area collimated to a field of view of $10' \times 3^\circ$ (FWHM) sensitive in the energy range 1.3–30 keV divided into 15 energy channels³.

The light curves of Cyg X-1, as observed in the medium energy range (1–7 keV) of the Utrecht detector and in the 1.3–7 keV window of the Cambridge detector, are presented in Fig. 1. The source is observed to be in a high intensity state, similar to the one observed⁴ before the transition in April 1971. The count rate has increased by a factor of 8–10 in the Utrecht detector and a factor of about 4 in the Cambridge detector compared with the observations on Cyg X-1 made with ANS in November 1974 (our unpublished work with W. Forman, C. Jones and

Y. Tanaka). The count rate in the first measurement on May 1 is at the lowest point for both detectors, at 80 counts s^{-1} and 58 counts s^{-1} respectively, and rises to 100 counts s^{-1} (80 counts s^{-1}) on May 3, suggesting that the source is in its last stage of the actual transition. After May 3, the intensity averages above 100 counts s^{-1} (80 counts s^{-1}) with excursions up to 160 counts s^{-1} (130 counts s^{-1}). The spectrum is in all data very steep, similar to the one observed⁴ before April 1971. In fitting the measured pulse height spectrum with a source spectrum of the form

$$dF/dE = AE^{-\alpha} \exp(-\sigma n_H) \text{ keV}(\text{cm}^2 \text{ s keV})^{-1}$$

where σ is the Brown and Gould⁵ interstellar absorption and E the photon energy in keV, we find a best fit for the first measurement on May 1 of $\alpha = 2.6 \pm 0.2$ and n_H (equivalent) $= 7.1^{+3.0}_{-1.0} \times 10^{21} \text{ atoms cm}^{-2}$. In general, all the measurements fit with a power-law energy index range in the narrow band of 2.2–2.8, with an absorption of $8.4^{+4.0}_{-2.0} \times 10^{21} \text{ atoms cm}^{-2}$, whereas in the November 1974 data the spectrum was as flat as $\alpha = 0.4$, changing on a time scale of minutes to $\alpha = -0.5$ (a positive slope). The beryllium window counters also show little if any change in the intensity and spectrum above 8 keV, as compared to the ANS observations of November 1974.

The polypropylene counter–parabolic mirror combination has a transmission window between 0.2 and 0.28 keV and a side lobe between 0.4 and 0.7 keV. The pulse height discriminator levels are optimised for 0.2 to 0.28 keV, and in view of detector resolution set to 0.13 and 0.41 keV. The small efficiency at 0.4–0.7 keV is due to the finite counter resolution in the 0.13–0.41 keV pulse height interval. By integrating the Cyg X-1 signal in the parabolic section over 1,600 s, we observe a count rate of $0.38 \pm 0.04 \text{ counts s}^{-1}$, which in view of the large column density of interstellar matter to the source must be due to the high energy end of the detector window. The interstellar absorption for $7 \times 10^{21} \text{ atoms cm}^{-2}$, assuming Brown and Gould⁵

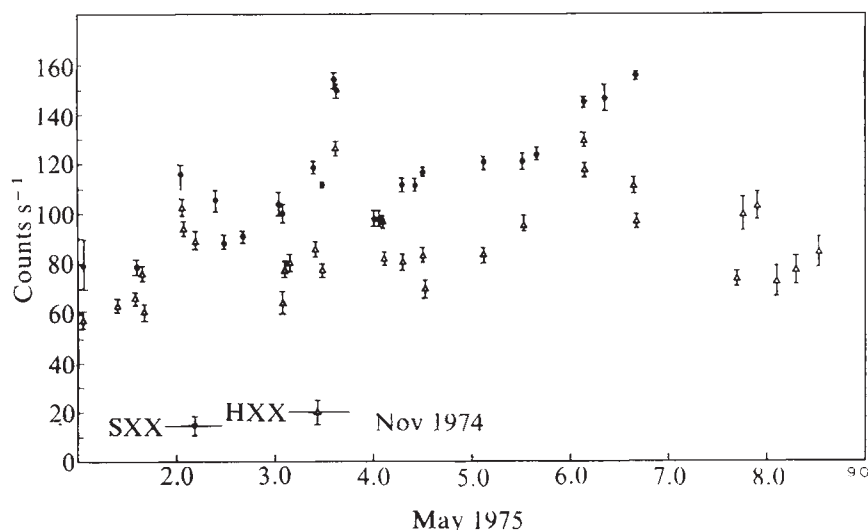


Fig. 1 The preliminary light curve of Cyg X-1 between May 1 and May 8, 1975. Black dots indicate the count rate of the Utrecht medium energy detector SXX (1–7 keV), open triangles are the count rates of the Cambridge hard X-ray detectors HXX (1.3–30 keV). The average count rate during the November 1974 observations are also indicated. Bars indicated typical excursions in intensity during observations of a few minutes. Marker on date axis indicates 0000 UT for indicated day.

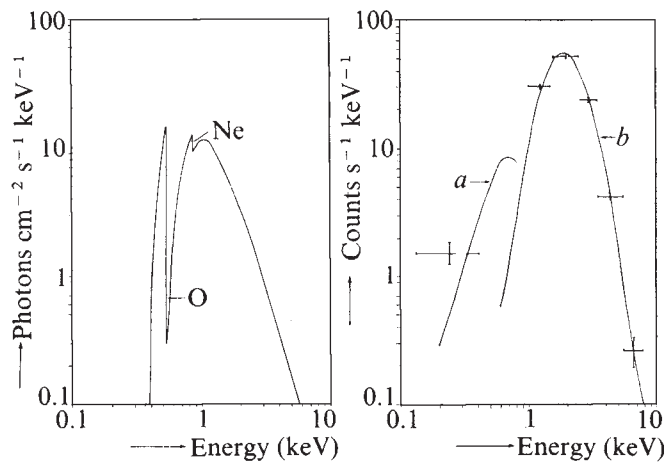


Fig. 2 Spectrum of Cyg X-1. The left-hand side shows the flux at Earth for a typical set of best fit parameters. O and Ne mark the absorption edges. The right-hand side shows this spectrum convolved with the detector response of the two Utrecht detectors and the observed count rates in the different pulse height channels. The signal in the polypropylene counter (a) is integrated over a longer time interval than that of the Ti counter (b).

abundances, is 3.3 mean free paths just below the oxygen absorption edge of 0.53 keV, and 19 mean free paths at 0.28 keV. The ultraviolet flux of the ninth magnitude B0I stellar companion of Cyg X-1 is three orders of magnitude too low to explain the soft flux by contamination of the ultraviolet flux, both from the theoretical point of view and from the measured ultraviolet spectrum⁸. The detected flux in the soft X-ray counter is entirely consistent with an assumed source spectrum with power law (energy) index of 2.5 and interstellar absorption of 7×10^{21} atoms cm^{-2} . The expected count rate for this spectrum is 0.28 s^{-1} . This spectrum is presented in Fig. 2, together with the measured flux and the spectrum convolved with the response of the two detectors. With this spectrum the source intensity at 0.50 keV then corresponds to 13 ± 3 photons $\text{cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$ and the power spectrum is valid over the entire range from 0.5 to 6 keV (ref. 7).

The intensity and spectrum of X radiation from Cyg X-1 observed by the ANS instruments are now dramatically different from those observed by the same instruments during November 1974. The present data, both the high intensity and the changed spectra, are consistent with what was reported by Uhuru for the 'high' state of 1970–71. For this reason, and because this high level has persisted for about six days as we see it, we conclude that Cyg X-1 has reverted to its high state. Our data also suggest that the transition was in progress when we began observing on May 1.

Several descriptions have been put forward to explain the observed low and high intensity states of Cyg X-1 on the basis of accretion disk models⁹. In these models the flux above 10 keV originates in the optically thin inner disk, and the flux below 10 keV in the optically thick region immediately surrounding the inner disk. The transition could be due to a change in accretion rate or to the switch on and off of a secular (Lightman and Erdlich) instability in the inner region of the accretion disk⁹ where the disk could become spatially thick and optically thin wherever radiation pressure dominates gas pressure. Thorne and Price⁸ make various predictions for the photon energy E_{max} at which the source spectrum exhibits a maximum.

From the spectrum presented in this paper $E_{\text{max}} < 0.5 \text{ keV}$ suggesting, on the basis of the qualitative model of Thorne and Price, that the transition is triggered by a change in

the accretion rate in a disk model where the optically thin region is indeed extended (Lightman–Erdlich instability operative).

The power spectrum of Cyg X-1 integrated from 0.5 to 6 keV leads to an intrinsic luminosity at 2.5 kpc ranging between 7.0×10^{37} and $9.6 \times 10^{37} \text{ erg s}^{-1}$.

Extended X-ray, optical and radio measurements are now in progress and may help clarify the nature of this transition. On May 9 the radio emission was several times higher than at any time in the previous four years¹⁰. This is in contrast to the behaviour during the previous high state when Cyg X-1 was not seen in radio. Also, Bolton¹¹ has not seen any spectroscopic changes in the optical emission.

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Observations of a new transition in the emission from Cyg X-1

THE all-sky X-ray monitor aboard Ariel V is a pinhole camera sensitive to X rays in the energy range 3–6 keV. It has an effective area of 0.6 cm^2 with a duty cycle of $\sim 1\%$ for a given source, and monitors more than 3π of the celestial sphere each orbit¹.

Following the first report² that the emission from Cyg X-1 had increased, we used data from the all-sky monitor to establish that a sudden transition had occurred on April 22, 1975, 9 d before the initial observation from ANS. Cyg X-1 has been observed almost continuously since $\sim$ November 1, 1974, by this experiment, and has been relatively constant in apparent magnitude until the reported effect. Its pre-transition average intensity was 0.5 ± 0.1 efficiency-corrected photons $\text{cm}^{-2} \text{ s}^{-1}$ in the energy range 3–6 keV, where the 20% error is the estimated absolute calibration uncertainty. During the > 5 months before the transition, the average daily intensity was

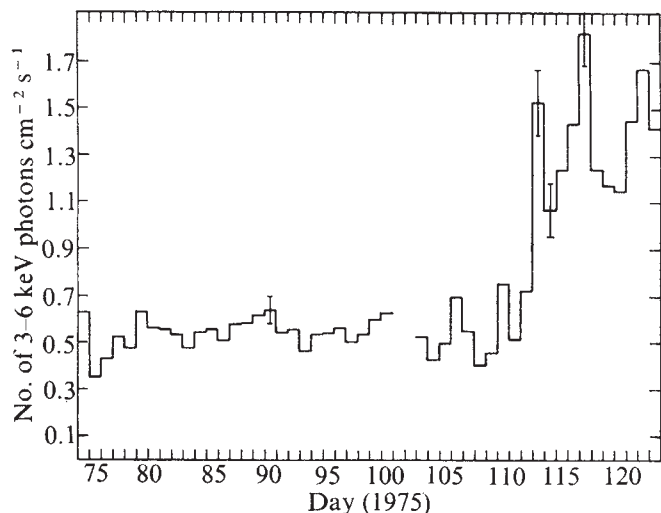


Fig. 1 Daily average intensity of Cyg X-1 for 50 d. The constancy of the intensity before day 112 is representative of the source (as measured by this experiment) since November 1974.

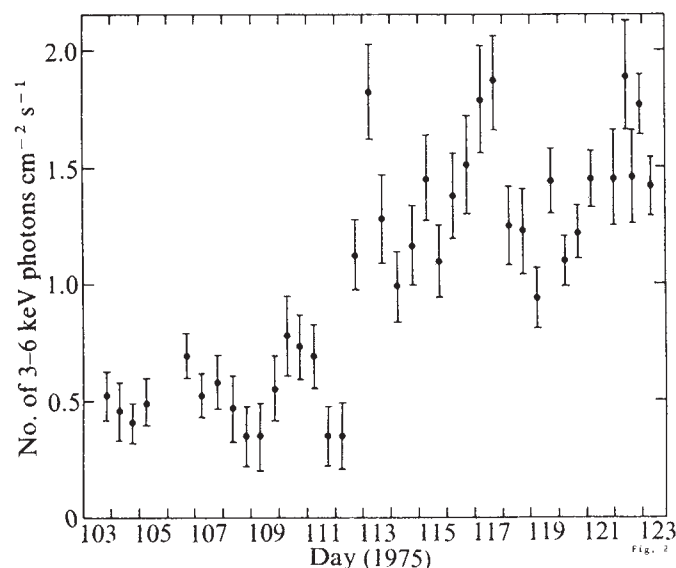
rarely measured as high as $0.7 \text{ cm}^{-2} \text{ s}^{-1}$. The Crab Nebula intensity in the same units is $1.4 \text{ cm}^{-2} \text{ s}^{-1}$.

Daily averages between March 15, 1975 (day 74), and May 3, 1975 (day 123), are displayed in Fig. 1. No Cyg X-1 data were taken during days 101 and 102, but the data taken up to day 100 are representative of the behaviour of Cyg X-1 since the onset of experimental operation. From days 103–123 Cyg X-1 was located at approximately the same spacecraft latitude, so that no intensity changes may be ascribed to systematic errors in the data analysis. On day 123 the satellite was reoriented to allow the four Ariel-V experiments which view along the spin axis to observe Cyg X-1, placing it outside the field of view of this experiment.

The data after day 103 are shown with an approximately half-day resolution in Fig. 2. The natural resolution of the experiment is one satellite orbit (~ 100 min), but the statistical errors on some data points then become more than half as large as the magnitudes, so that we have not attempted to present finer resolution than that shown in Fig. 2.

Clearly, the rise above $1 \text{ cm}^{-2} \text{ s}^{-1}$ during the latter half of day 112 is real, as the intensity continues to rise to more than three

Fig. 2 Intensity measured from Cyg X-1 over approximately half-day accumulation times. The source is no longer in the field of view of the experiment after day 123 (May 3).



times the pre-transition average value during the first half of day 113. The intensity then seems to oscillate between ~ 1 and $\sim 2 \text{ cm}^{-2} \text{ s}^{-1}$ with a time scale of several days until it passes out of our field of view on day 123 (May 3, 1975). As the experiment has no spectral resolution, we cannot comment on any spectral change which may have accompanied the intensity transition. The 3–6 keV acceptance window is relatively insensitive to spectral shape, however, so that the indicated intensities in the 3–6 keV band should be correct whether or not the spectrum changes.

Cyg X-1 has remained remarkably constant in intensity and spectrum³ since the “transition” in 1971 (ref. 4). Before 1971, the intensity was extremely variable, and the spectrum was always softer than that measured afterwards. Whether or not the effect reported here represents a long term return to its pretransition state is something which this experiment will be able to monitor in the future.

Millisecond temporal structure in the emission from this source has previously been measured only by large area rocket-borne experiments⁵, and the present intensity increase should now make possible its study with instruments of smaller area mounted on various satellites. L.J.K. acknowledges support from the University of Maryland.

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Ariel V and Copernicus measurements of the X-ray variability of Cyg X-1

THE collimated proportional counter (experiment C of MSSL) on Ariel V viewed Cyg X-1 in 1975 from May 3 1400 UT until May 15 1000 UT. We report here observations of the intensity and spectrum obtained with an integration time of ~ 30 min. They show that, at the beginning of this period, Cyg X-1 was more intense, had a steeper spectrum and was generally more active than has been observed during the past $2\frac{1}{2}$ yr by Copernicus^{1,2}. Towards the end of the period the source seemed to be returning to its lower state.

Figure 1 shows the 1.5–15 keV and the 2–6 keV fluxes from Cyg X-1 during the Ariel V observations. The dominant errors in the values of intensity are due to uncertainty in the total integration time, rather than statistical errors, and it is the total $\pm 1\sigma$ errors which are shown on the two intensity light curves. Also plotted against time is the photon index of the power law spectrum obtained by fitting the data in the 1.5–15 keV energy range. The values observed (with Ariel V) during a brief look in October 1974 are also indicated.

The intensity and spectral index were indicative of a high state⁴ at the start of the observation on May 3, 2 d after the onset of the low-high transition reported³ from ANS. The source remained in the high state until May 9 or 10; it then began to recover its low state values represented by the October 1974 data. The transition back to

Table 1 2-10 keV X-ray observations of Cyg X-1

Date	Measurement	Ref	Photon power law spectral index	State of source
June 1964	Rocket (NRL)	5	Not given	High (based on intensity)
April 1965	Rocket (NRL)	6	Not given	Low (based on intensity)
October 1965	Rocket (ILL)	7	Not given	Low (based on intensity)
October 1966	Rocket (AS&E)	8	-1.7 ± 0.1	Low
September 1967	Rocket (NRL)	9	-1.5 ± 0.1	Low
September 1970	Rocket (GSFC)	10	-2.6 ± 0.3	High
November 1970	Rocket (MSSL)	11	-2.8 ± 0.2	High
December 1970 to March 1971	Uhuru	4	-4.05 ± 0.14	High
April 1971 to May 1972	Uhuru	4	-1.45 ± 0.03	Low
October 1972 to September 1974	Copernicus	1, 2 and this paper	-1.6 ± 0.1	Low
October 1974	Ariel V	This paper	-1.7 ± 0.2	Low
May 1975	ANS	14	-3.5 ± 0.2	High
May 1975	Ariel V	This paper	-4.2 ± 0.2 to -2.1 ± 0.2	High, then approaching low
May 1975	Copernicus	This paper	-2.1 ± 0.2	Approaching low

the low state was not complete when the satellite moved on in its observing programme on May 15.

Figure 2 shows the measured spectrum and best fit power laws of two typical orbits at the beginning and end of this run of data. The spectrum of May 6 shows an excess of high energy X rays above the power law spectrum, as indicated by the higher value of χ^2 .

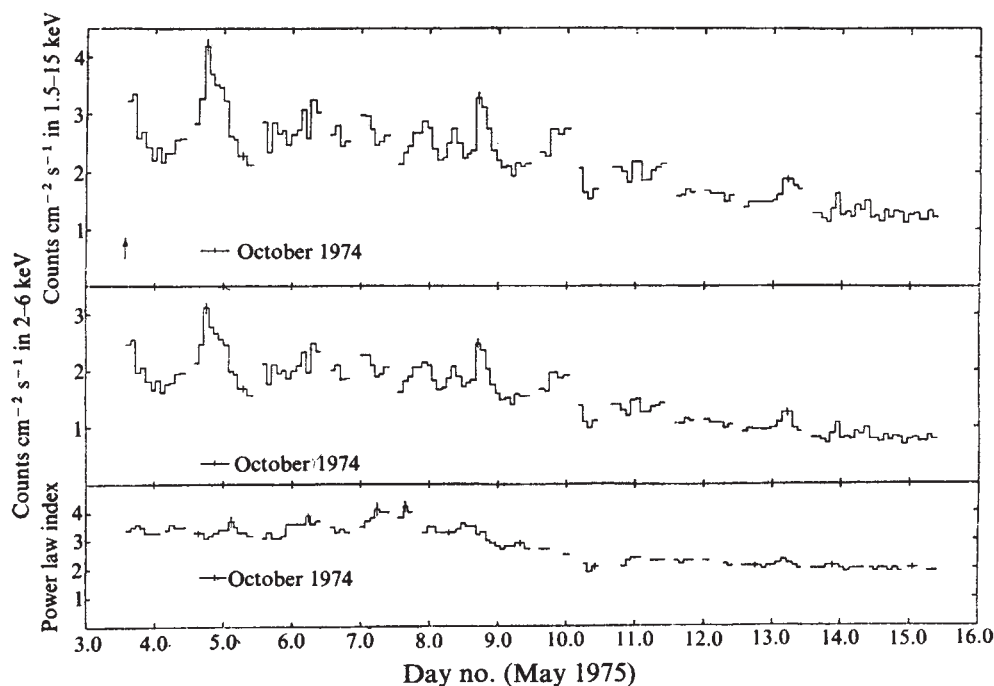
The high and low states seen by Ariel V are similar in intensity and spectral index to those reported from Uhuru⁴ before and after the down transition about April 1, 1971. The high energy excess in Fig. 2a (in the high state) is also seen in the Uhuru data before this transition, but the Ariel V spectrum does not have the sharp change of slope reported from Uhuru⁴.

Copernicus has observed Cyg X-1 on ten occasions between November 6, 1972, and May 12, 1975, of which the first seven are summarised by Mason *et al.*². A measurement of spectral index on May 12, 1975, by Copernicus agrees with the value -2.1 ± 0.2 measured then by Ariel V. On all the nine previous occasions the slope was significantly different at -1.6 ± 0.1 .

The implication is that Cyg X-1 was in its low state from April 1, 1971, until May 1, 1975, and in a high state for about 20 d thereafter. (If, however, 20–30 d is a typical duration for the high state, such flashes could slip between gaps in the Copernicus coverage, which are up to 180 d long.)

Table 1 lists spectral indices in the energy range 2–10 keV of Cyg X-1 taken from the literature. The first measurement of a spectral index considerably different from $\alpha = -1.6$ was reported by Bleach *et al.*¹⁰ on September 21, 1970. Subsequently data from Uhuru⁴ also yielded a departure from the usual value of the spectral index, as did a measurement by Cruise¹¹, made on November 11, 1970, which showed $\alpha = -2.8$. It is possible that this high state, characterised by enhanced emission in the 2–10 keV region, together with a steeper and more variable spectrum, lasted at least six months until the down transition reported by Tananbaum *et al.*⁴. It is improbable but not impossible for an up state of this duration to have occurred during the Copernicus measurements without having been detected. It seems that the low state, characterised by the power law spectral index stable at -1.6 , is the usual state of Cyg X-1 in which the source remains for about 90% of the time.

We have searched the Copernicus data taken between 1972 and 1975 for any systematic change in X-ray behaviour which might herald a transition. One possibility is a variation in the orbital phase at which absorption events are seen². The data of Mason *et al.*² suggest a systematic change in phase of the absorption events on a time scale of a few years (their Fig. 3), and this trend is confirmed by two further observations: Li and Clark¹² observed, with OSO 7, an absorption event in June 1973 at

**Fig. 1** The X-ray intensity and spectral index for the May 1975 observations with Ariel V.

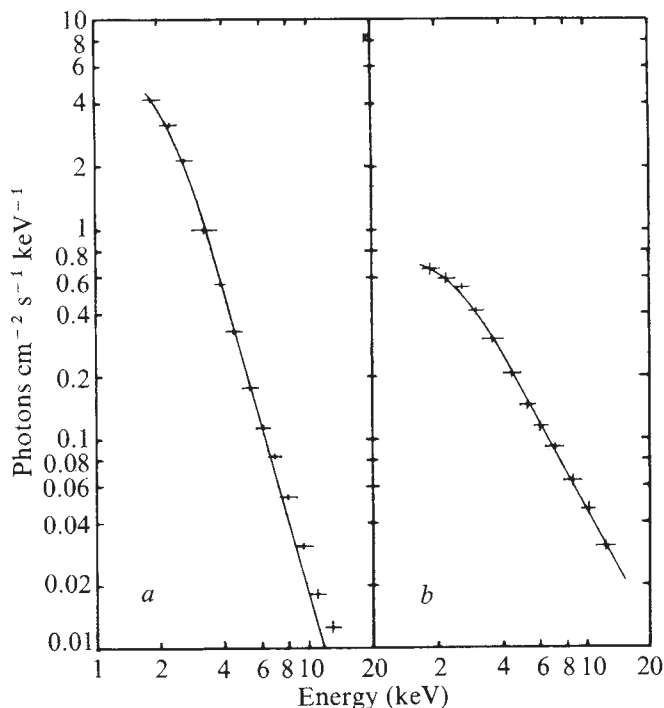


Fig. 2 Power law spectra obtained on two representative orbits. *a*, May 6; *b*, May 13. For curve *a* (orbit 3080), $N(E) \propto E^{-3.6 \pm 0.1}$; curve *b* (orbit 3191), $N(E) \propto E^{-2.0 \pm 0.05}$. χ^2 per degree of freedom is 4.7 for May 6 and 1.8 for May 13.

a phase of $+0.053$ using the ephemeris of ref. 2; and in October 1974 a Copernicus observation revealed an event at phase -0.12 . The project orbital phases of the events at the time of the 1971 and 1975 transitions are $+0.20 \pm 0.02$ and -0.18 ± 0.01 respectively, which, within the errors, is symmetrical about the line of centres of the binary system.

Without a knowledge of the behaviour of the absorption events following the May 1975 transition, we can only draw attention to this coincidence. But if the absorption events are, as suggested in ref. 2, a manifestation of the gas flow which powers the X-ray source, a correlation between their behaviour and periods of activity is not unreasonable, since changes in the accretion process are a possible trigger mechanism for the transition¹³.

This hypothesis does predict that the phase or nature of the absorption events will change significantly following the May 1975 outburst.

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Another major change in the radio source associated with Cyg X-1

THE sudden appearance^{1,2} of a faint radio source inside the probable position region for the X-ray source Cyg X-1 was associated with major changes in the X-ray emission observed³ during the period February–May 1971. This coincidence established a probable association with the X-ray source. The accurate position of the radio source led to an identification^{2,4} with the bright, blue star HDE226868. Subsequent optical investigations have established^{5,6} this object to be a binary system with a massive, invisible component that may be a black hole.

Since 1971, no major changes in the Cyg X-1 X-ray and radio source had been reported until the spring of 1975.

We now report further observations of the Cyg X-1 radio source, the most recent of which were made after learning⁷ that Cyg X-1 had undergone another major change in X-ray state in, or before, early May 1975. The radio observations were made with the NRAO interferometer at 2,695 and 8,085 MHz and are shown in three parts in Fig. 1. The first part has been published previously⁸, but is shown again for completeness and comparison. It shows the 1971 X-ray transition in the 2–6 keV range and the initial appearance of the radio source between March 22 and March 31, 1971, followed by the radio history of the source until October 1972. The second part shows observations between March 1973 and March 1975, which have not been published before. The last part shows observations taken during May 1975 after learning of the recent change in X-ray state⁷.

An extensive discussion of the 1971–1972 radio data has been given by Hjellming⁸. The major point of interest is the approximate mean level of 0.015 Jy maintained by the radio source at both 2,695 and 8,085 MHz during the period March 1971 to March 1975. This level is shown by dashed lines in Fig. 1. There are some clear fluctuations about this norm, particularly during extensive observations in October 1972, but a mean level of 0.015 Jy was maintained by the radio source over a time span of four years. The observations of Cyg X-1 at 1,415 MHz by Braes and Miley^{2,9} also showed a mean flux level of 0.015 MHz. Thus one of the major characteristics of the Cyg X-1 radio source between March 31, 1971, and March 1975 is a flat radio spectrum, that is, a spectral index of 0.

The Cyg X-1 radio observations on May 9, 1975, are shown on a greatly expanded scale in Fig. 1, because, in addition to the fact that the mean flux density for this day was 0.035 Jy at 8,085 MHz, considerably above the previous norm, the variations from a peak level of 0.045 Jy to a lower level of 0.030 Jy over a period of 5 h on May 9 are real. Subsequent sampling of the radio source between May 10 and May 20 is shown in Fig. 1, plotted on a time scale intermediate between that of the May 9 data and the earlier data.

The mean daily levels of Cyg X-1 during the period May 9–20, 1975, are 0.031 Jy at 8,085 MHz, and 0.002 Jy at 2,695 MHz. These levels clearly represent a new state for the radio source. A particularly interesting feature of the new state is the change of the mean spectral index from 0 to 0.31. This means that if the radio emission mechanism is thermal, the source has become slightly optically thick

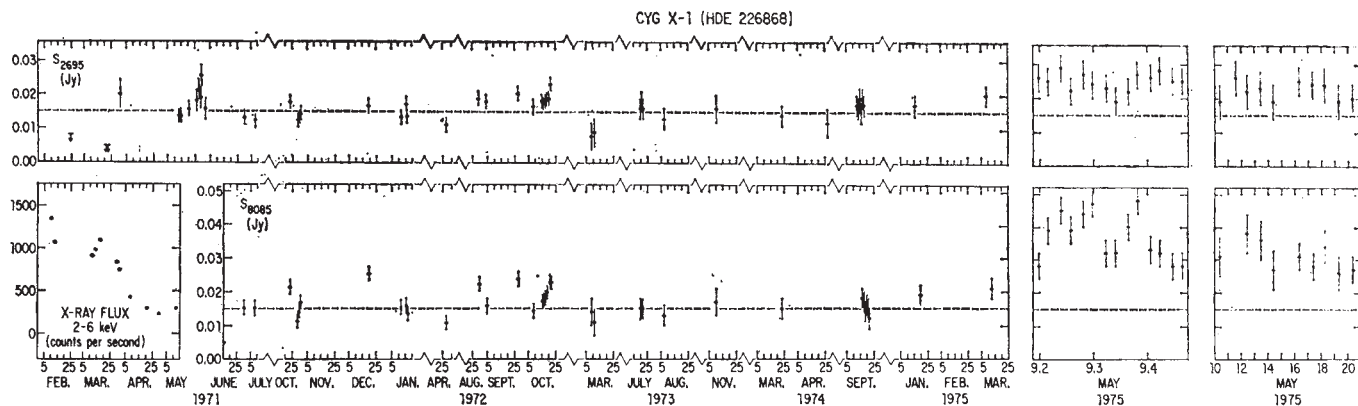


Fig. 1 Radio observations of Cyg X-1 (HDE226868) at 2,695 and 8,085 MHz are plotted as a function of time for the period between February 1971 and May 1975. Also shown is the change in X-ray level in the 2-6 keV energy range during February-April 1971. Note that the time scale for the abscissa is not linear and that different time scales are used in plotting the May 1975 observations.

with an optical depth of 0.17 at 8,085 MHz; however, if the radio emission mechanism is non-thermal, the source is self-absorbed.

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Can soft γ -ray bursts be emitted by accreting black holes?

THERE are many models for the observed γ -ray bursts¹, but none has proved entirely satisfactory. Here we suggest another possible model: that the γ rays are produced by inverse Compton processes occurring deep in the ergosphere of a fast rotating, accreting, black hole. At the time of burst, plasma, which has an optical depth of the order of the relevant gravitational distances, emerges near the horizon due to an instability, and free falling photons, which are produced by viscous processes in an accretion disk further away, are being scattered off it. In this model the black hole itself is probably a member of a binary system, and, depending on its steady-state accretion rate, may or may not be observable as an X-ray source.

With Katz, we have shown² that photons, which free-fall into a Kerr horizon to be Compton scattered by tangentially moving electrons, can acquire very high energies by the Penrose mechanism. The scattered electrons share their negative energy with the protons; thus, the excess photon energy may be supplied mainly by plasma gravitational energy. When the scattered photons escape to infinity, their energies at infinity do not exceed a few $m_e c^2$. We have calculated numerically overall energy spectra for several realistic scenarios of plasmas with

turning points close to the horizon, by the Monte Carlo procedure. Details will be published elsewhere; here we report briefly the emerging qualitative picture.

The maximum energy obtained in the conversion of free-falling X-ray photons into γ rays, increases as the collision point approaches r_{mb} , and can become as high as a few electron masses for $a/M \rightarrow 1$. For a canonical black hole³ ($a/M = 0.998$), and for an X-ray photon of 10 keV falling in radially, energies as great as 600 keV can be reached; with a 50 keV photon falling in radially, energies reach 900 keV. This maximum energy increases when collisions of outgoing photons are considered and, at any rate, increases rapidly as a/M grows above its canonical value.

A small number of collisions help to increase the overall efficiencies by directing additional scattered photons outwards. But when many collisions occur, the photons merely become "thermalised", and the possibility of using free-fall no longer exists. The maximum efficiency was of the order of 10% or higher in the examples considered, and one may also expect a certain anisotropy in the emitted spectra. All our spectra seem to stabilise at spectra similar to those observed⁴ when collisions occur deep enough and rotation is fast enough.

The inner disk region is secularly unstable^{6,7} and is, therefore, a likely location of matter flow instabilities. The time scale for these instabilities is of the order of a few seconds or less, whereas the time scale for fluctuations around and inside r_{ms} is of the order of a few milliseconds. For accretion rates which are not larger than $10^{-3} M_{\odot} \text{ yr}^{-1}$, and for canonical black holes with masses $\sim 10 M_{\odot}$, the number of (proper) optical depths for high energy photons in the region between r_{ph} and r_{ms} is normally less than one. The suggested γ -ray production mechanism will only be effective for a depth of 1-3 optical paths, which is not an inconceivable number for instabilities in such disks, but is an unlikely number for steady-state flow. This is also in agreement with recent disk structure models (S. L. Shapiro, A. P. Lightman and D. M. Eardly, unpublished and A. P. Lightman, personal communication.)

The initial photons could come from the steady-state flux of the disk or, which seems more likely, from the event itself; one could imagine a sudden burst of matter flow on the companion star⁸ to dump a lot of material on the disk, thus producing both a large number of X-ray photons and the necessary deep ergosphere plasma flow. The occurrence of softer, and longer lasting, X-ray bursts, on which some of the γ -ray events seem to ride⁹, is certainly not inconsistent with such an interpretation. The Vela satellites' γ -ray burst 71-2 (ref. 10), which seems to have come from the general direction of Cyg X-1, occurred close to a remarkable transition in that X-ray source⁵. It is tempting to associate that burst with a disk instability, which is, in turn,

also indicated by the X-ray and radio data (ref. 11 and S. L. Shapiro, A. P. Lightman and D. M. Eardly, unpublished).

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Mother and baby paradox

THE 'twin paradox' of relativity theory describes the difference in the elapsed time between the twin who stays at home and the one who goes on a space flight and returns. No genuine paradox is involved; elapsed time is not in general independent of the state of motion. In this case the difference in the elapsed time is related to a difference in the (locally measurable) acceleration histories of the twins; the space traveller must suffer an acceleration, however brief, in order to return.

But no paradox is involved, even if there is no locally measurable effect to distinguish the two timekeepers. General relativity provides such possibilities, one of which I describe here.

A mother, desiring her baby to remain young for as long as possible, decided on the following plan of action. Each night, when the baby was asleep, she went out and collected masses from afar, symmetrically, and arranged them in a spherical shell around the baby's crib (herself staying outside and some distance away from the completed shell). The baby's sleep was not disturbed, no acceleration being measurable inside the shell. In the morning before the baby woke up, she dismantled the shell and stored the masses again far away. Doing this carefully, with spherical symmetry, again no acceleration could be felt by the baby. But, in the 12-h period of the night experienced by the mother, the baby had aged only $12 \text{ h} \times (1 - GM/Rc^2)$ (for small values of GM/Rc^2 , where M and R refer to the mass and radius of the shell, and G and c are the gravitational constant and speed of light respectively). In the daytime the components of the shell were stored so far apart that the large value of R made the value of GM/Rc^2 negligibly small.

By this procedure the mother's and her baby's timekeeping were caused to be different; yet, unlike the case of the 'twin paradox' of special relativity, without any necessity for a different acceleration history.

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Trapped helium and argon and the formation of the atmosphere by degassing

I REPORT here some radiogenic ^4He and ^{40}Ar data measured in the glass margins of deep sea pillow basalts, and comment on their applicability to discussions of formation of the Earth's atmosphere by degassing from a solid Earth.

Discussions of such degassing generally assume either catastrophic or continuous degassing (see refs 1, 2 and references therein). In fact, there is no reason why these two sets of models should be considered to be mutually exclusive. The history of the Earth may include both a catastrophic event with an associated 'big burp', and later continuous degassing.

Ozima and Kudo² and Ozima³ have presented calculations which indicate the evolution of the terrestrial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio as a function of time, in either catastrophic or continuous degassing models of atmospheric formation. In particular, Ozima³ has shown that the present $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the mantle is a sensitive indicator of the validity of the catastrophic model. In such models, the degassing is defined by any two of these four quantities: The time of degassing; the fraction of Ar degassed; the K content of the Earth; the present value of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio within the Earth. Ozima showed sets of graphical solutions for these quantities which satisfy the total Ar abundance and the present atmospheric ratio of 295.5.

The rare gases found in deep sea basaltic glasses are clearly those trapped within the rock on its eruption⁴⁻⁶, due to the high hydrostatic pressures and rapid chilling, and as such represent mantle-ambient gases. A characteristic of those trapped gases is the presence of "excess" radiogenic ^4He and ^{40}Ar , in amounts greater than can be accounted for by the decay of U, Th and K subsequent to their eruption⁴⁻⁷. Similar "excess" ^{40}Ar contents have been measured in ultramafic intrusives⁸ and in continental rock, but their origin is not as clear.

Ozima therefore accepted the $^{40}\text{Ar}/^{36}\text{Ar}$ trapped in submarine basalts as representing the isotopic ratio in the upper mantle; the published ratio available to him, $\sim 1,000$, could not be reconciled with a catastrophic degassing model of the Earth's atmosphere without the assumption of an unacceptably high K content³. He noted, however, following Schwartzman¹⁶, that if the ultramafic values for this ratio ($\sim 10,000$) were indicative of mantle gases, then the catastrophic model could not be ruled out. The $^{40}\text{Ar}/^{36}\text{Ar}$ values shown here in Table 1, measured in the glassy margins of deep sea basalts from both the Atlantic and Pacific Oceans, on both ridge and non-ridge samples, reach values as great as $\sim 15,000$. If these values are accepted, Ozima's argument against the catastrophic model must be relinquished. The argument can be replaced, however, with one based on the $^4\text{He}/^{40}\text{Ar}$ data of Table 1.

Schwartzman predicted a $^4\text{He}/^{40}\text{Ar}$ ratio in the present-day mantle of between 1.36 and 2.23, depending on the

Table 1 ^4He , ^{40}Ar , and ^{36}Ar data in glassy margins of deep sea basalts. The rocks are described in refs 4 and 17. All units are 10^{-8} s.c.c. g^{-1} . The ^{40}Ar reported is total measured ^{40}Ar ; correcting for atmospheric contamination does not change the $^4\text{He}/^{40}\text{Ar}$ results significantly

Sample	^{40}Ar	^{36}Ar	^4He	$^{40}\text{Ar}/^{36}\text{Ar}$	$^4\text{He}/^{40}\text{Ar}$
Vema Fracture Zone, Atlantic Ocean					
72	218	0.0175	—	12,500	—
	77	0.024	1,150	3,210	15
	74	0.0167	1,300	4,430	17
88	290	0.055	2,400	5,200	8.3
	330	0.075	2,400	4,400	7.3
92	235	0.025	1,600	9,400	6.8
	204	0.028	2,370	7,300	12
East Pacific Rise, $\sim 10^\circ\text{S}$					
32	62	0.0174	84	3,600	1.35
	81	0.067	180	1,210	2.2
40	81	0.0104	1,250	7,800	15
	38	0.032	500	1,200	13
44	210	0.0133	1,800	15,800	8.6
	250	0.016	1,970	15,600	7.9
East Pacific Seamount, $\sim 10^\circ\text{S}$ — 98°W					
50	157	0.042	2,800	3,740	18

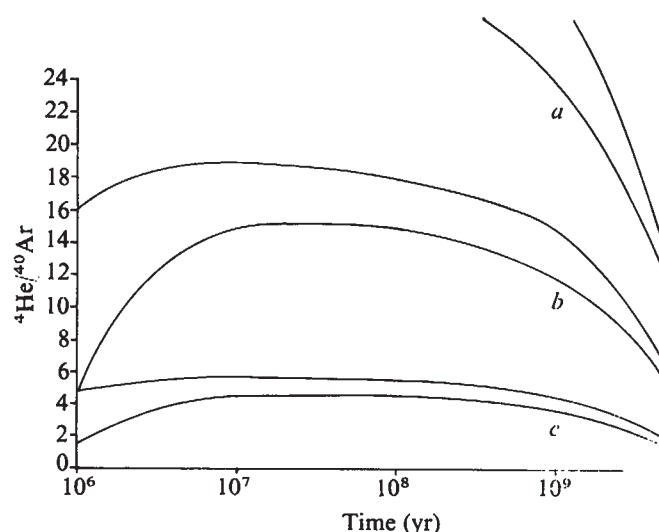


Fig. 1 $^4\text{He}/^{40}\text{Ar}$ ratio as a function of elapsed time in a closed system, for various values of the K/U and Th/U ratios.

- | | | |
|----|------------|---------------------------|
| a, | Th/U = 3.6 | } K/U = 1.5×10^3 |
| b, | Th/U = 2 | |
| | Th/U = 3.6 | } K/U = 3×10^3 |
| | Th/U = 2 | |
| c, | Th/U = 3.6 | } K/U = 10^4 |
| | Th/U = 2 | |

Th/U ratio, for a catastrophic degassing model with very early degassing'. He noted that this is in agreement with a ratio estimated for the former environment of ilmenite nodules from Hawaiian nepheline melilitite basalt⁹, and pointed out that greater values (2–10) found by Wasserburg *et al.*¹⁰ in volcanic gases might well be due to preferential leaking of ^4He from wall rocks. But some of the values shown here (Table 1) are much too great for such a model, and the preferential leaking argument is much weaker for sub-oceanic mantle sources than for the terrestrial volcanic emanations of Wasserburg *et al.* Since the gas trapping mechanism cannot be 100% efficient, and since in any gas loss ^4He will be lost preferentially to ^{40}Ar , the largest $^4\text{He}/^{40}\text{Ar}$ ratios reported here are minimum values for the magma-ambient ratios. These values, 15–18, cannot be accounted for at all if the magmatic K/U ratio is similar to the crustal value of 10^4 , even less if the ratio is chondritic ($3\text{--}6 \times 10^4$), as seen in Fig. 1. Thus the data rule out all models which ascribe the terrestrial atmosphere to any type of degassing of a mantle which is chondritic or crustal with respect to the K/U ratio.

K/U ratios measured on ultramafic rocks lead to an estimated mantle value of $\sim 3 \times 10^3$ (refs 11–14). Assuming this value gives maximum $^4\text{He}/^{40}\text{Ar}$ values of 15–20 (Fig. 1), but only for an age $\lesssim 10^9$ yr; for a catastrophic degassing model this is the age of the catastrophe, and is much too young. The catastrophe is normally identified with formation of the Earth's core, and must have occurred at least within the first 10^9 yr of Earth history; so the age must be $> 3.5 \times 10^9$. This necessitates a K/U ratio in the source region $< 1.5 \times 10^3$. Such a value is barely within the widest limits set by K/U measurements on ultramafic rocks: $1 \times 10^3\text{--}10 \times 10^3$ (ref. 12), $1 \times 10^3\text{--}5 \times 10^3$ (ref. 13). An even smaller K/U ratio would be necessary if core formation (and an associated 'big burp') is to have generated the atmosphere within the first 100 Myr of Earth history, as suggested by Oversby and Ringwood¹⁸.

These data do not suggest that catastrophic core formation, with or without an associated degassing 'burp', could not have occurred. They do suggest that the mantle has not been a closed system with respect to Ar and He

since any such postulated event, and therefore that significant later or continuous degassing from the interior Earth must have occurred. The relative contributions to our present atmosphere from a possible early catastrophic event and the necessary later degassing have yet to be worked out.

Further data on $^4\text{He}/^{40}\text{Ar}$ ratios in more submarine glasses of wider geographic location and of varying magmatic origins, and on K/U ratios in a wider variety of possible mantle materials, should settle the question conclusively.

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Geodynamic implications of transverse folding in the Western Alps for the Alpine fold belt

LARGE amplitude translations towards the external parts of the Alpine arc are still considered as the major feature of Alpine tectonics^{1–5}. More recent approaches, inspired by concepts of global tectonics^{6–8}, have led to the development of new models, none of which however accounts for the widespread distribution of early transverse folds which in general are thought to have been formed either before⁹ or later^{10–13} than the overthrusting, and are considered to be minor structures. New data on the variations in strike of both transverse and longitudinal folds have been gathered over wide areas of the Alps between Savone and the Simplon Pass. The results indicate that in the whole Pennine Zone as well as part of the external zone only two important successive phases of folding have occurred, the trends of which are completely different. Here I am concerned with the 'initial transverse phase' or 'main Alpine phase' of deformation.

The axes of folds generated during the early Alpine deformation, in each of the internal structural zones, now lie roughly in a radial position with respect to the alpine arc (Fig. 1). The S_1 primary cleavage is everywhere related to this phase of deformation. The following domains have been distinguished: (1) a zone of incipient slaty cleavage defined by illite-chlorite, for example in the uppermost overthrust sheets of the Briançonnais zone; (2) a zone of flow cleavage parallel to the axial planes of transverse isoclinal folds, for example in the Mesozoic cover of Vanoise¹¹ or in the Piemontese schistes lustrés of the lawsonite-glaucophane facies¹⁴; (3) a zone of foliation defined by the preferred orientation of phengite, glaucophane, zoisite, for example in the internal crystalline massifs and Piemontese

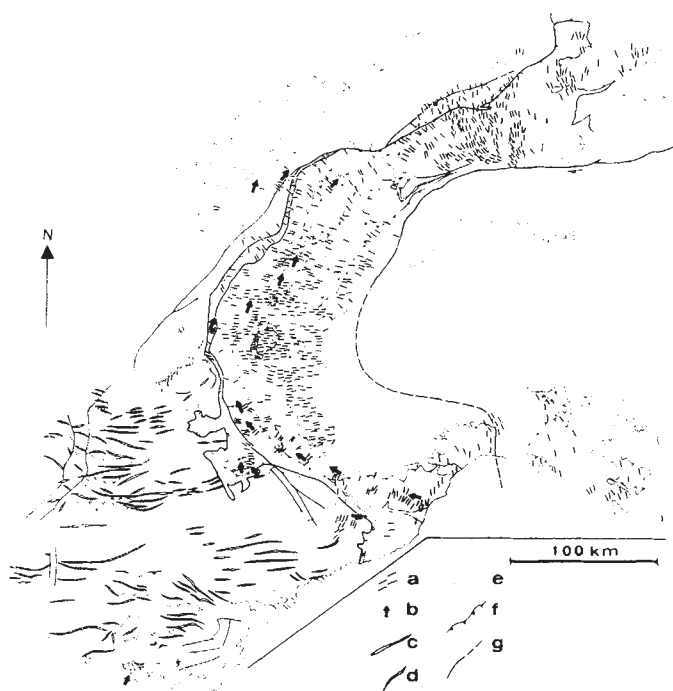


Fig. 1 Trend of transverse folds of the F_1 phase of Alpine deformation (post-Lower Eocene). In the Briançonnais and Grand St Bernard zones after Bertrand³⁴, Caron, *et al.*²², Ellenberger¹¹, Nicolas¹⁴, Vialon⁹, Wenk¹⁵, and Wunderlich³⁶, and personal field data in the Ligurian Alps, Briançonnais, the Aosta Valley and Valais. After Lemoine³⁷ for the External zone. a, Trend of F_1 transverse folds; b, sense of overturning of F_1 folds; c, pre-Senonian folds of the Devoluy; d, pyreneo-Provençal folds; e, northern limit of Pyreneo-Provençal folds; f, overthrust; g, strike-slip fault.

rocks of the eclogite facies; (4) a zone of foliation defined by biotite, sillimanite and anatectic mobilisates, exposed in the Barrovian metamorphic zones of the central Alps¹⁰ where a later high temperature-low pressure metamorphism is superimposed^{4,15} on an earlier high pressure metamorphism.

The style of the phase 1 folds differs depending on which structural zone they occur in.

Briançonnais cover with both internal and external carboniferous basement. No alpine deformation can be found previous to the phase 1 transverse folds. In many areas, the stratigraphic sequence and sedimentary features in the Lutetian flysch noir allow the sense of overturning of the folds to be clearly established (Fig. 1). On all scales, the transverse folds are similar in style and are syn-metamorphic. They are younger than the nummulitic flysch of lower to middle Eocene age. They were formed during the first large scale tangential movements in the Alps, the amplitude of which still remains unknown, under the

overburden of allochthonous Helminthoid flyschs (Upper Cretaceous age) originally emplaced as olistostromes in the Eocene sea.

Savoy sub-Briançonnais zone. The presence of recumbent to isoclinal refolded folds in this zone has been known for long time¹⁶. The steeply plunging folds which are overturned to the north were formed also during the first penetrative deformation of post-Eocene age.

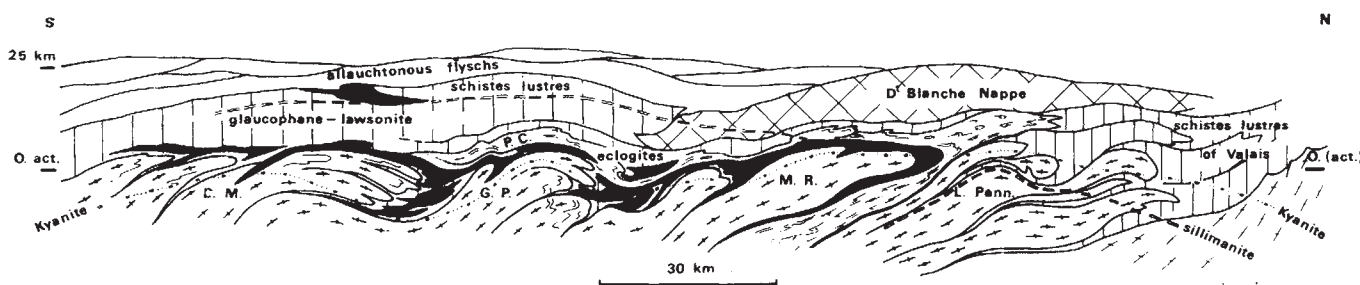
Piemontese schistes lustrés. The allochthonous character of the schistes lustrés overlying the internal crystalline basement rocks has been proved on the basis of stratigraphic and palaeontological evidence (substitution de couverture)¹¹ for the massifs of Vanoise, Ambin¹¹ and Acceglio¹⁷. The transverse folds have a similar style and trend in both the schistes lustrés and basement rocks^{9,11-13,18}. It is postulated that these syn-metamorphic folds are genetically related to the geosynclinal de nappes¹¹ or the obduction¹⁹ of Piemontese geosynclinal units of Mesozoic age²⁰ and remnants of oceanic crust²¹. The underlying heterogeneous basement rocks were subsequently rapidly buried under high pressure-low temperature metamorphic conditions, whereas the overburden required for high pressure-low temperature metamorphic conditions in the Piemontese units was represented by Austroalpine units, such as the Dent Blanche Nappe, or by overthrust sheets of ultra-Piemontese flysch.

In the areas where the S_1 cleavage or foliation is horizontal and unaffected by intense secondary penetrative deformation (Gran Paradiso, Dora Maira, Val d'Aoste), the original trend of the transverse folds has been preserved. But in many areas within the zone of the schistes lustrés, the second phase folds are also isoclinal where formed^{14,18,22,23} in calcareous schist units and gliding processes related to the secondary deformation have generated a considerable dispersion of the F_1 folds axes. In contrast, the F_2 slip directions show a more regular pattern²³.

The observations made on the very tight first phase folds which affect the meta-banded cherts of Queyras (Cottian Alps) in which the laminated bedding is still preserved, exclude the existence of an earlier penetrative deformation in the Piemontese zone, as recently suggested²³. F_1 folds in Piemontese rocks have possibly formed as a result of a long period of penetrative deformation (evidently starting in Upper Cretaceous times as suggested by recent dating of HP minerals²⁴) during translations of rock units under conditions of constant stress orientation.

In different metamorphic zones where such relations have been established, the high pressure-low temperature minerals are syn to late kinematic^{9-14,16,18,22,23,25,27} with respect to transverse folds. The metamorphic zonation is similar to other orogenic belts with alpine-type metamorphism²⁸. The width of each of the three principal metamorphic zones²⁹: (1) lawsonite $\pm$ jadeite zone; (2) zoisite-glaucofanite eclogite zone; (3) kyanite-eclogite zone, is only compatible with sub-horizontal isograds³⁰, now refolded in detail. Field evidence, for example west of Gran Paradiso, and between Monte Viso and the Briançonnais units, strongly suggests that the isograds plunge westwards and not eastwards, as recently postulated²⁸.

Fig. 2 North-south longitudinal combined section perpendicular to F_1 folds, showing the possible configuration of the internal crystalline massifs and the Piemontese obduction zone before the arc curvature. The total thickness of units is compatible with high pressure-low temperature or high pressure-high temperature Alpine metamorphism (after ref. 4).



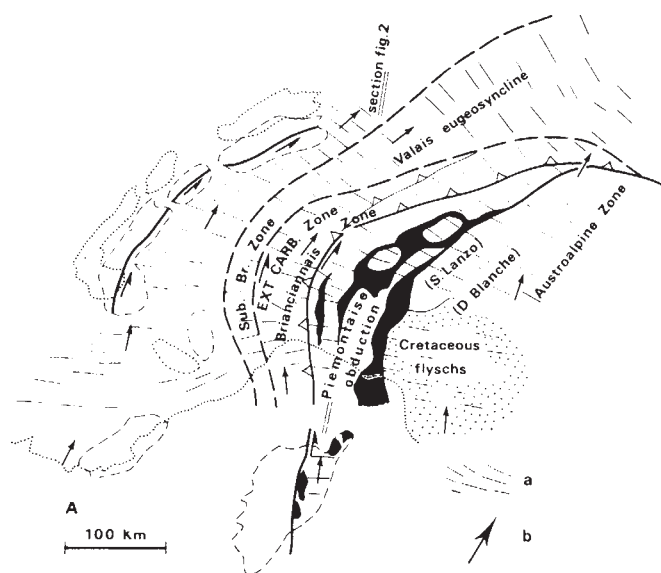


Fig. 3 Possible reconstruction of the western Alps before the arc curvature. The position of the Insubrian plate is the one postulated by Laubscher⁷. Minor rotations of external crystalline massifs are figured. The different structural zones are represented at their actual erosion level. Ophiolites are in black. a, Original trend of F_1 folds; b, direction of tectonic transport.

I would like to put forward a geodynamic model based on this evidence. The radial pattern of transverse folds with respect to the arc is a new factor that has been taken into account in tectogenetic models of the Alps. It seems unlikely that such a pattern could be primary as it would imply a single deformation field of curvature 180° .

In agreement with the data concerning the shortening generated after the F_1 deformation, it seems more likely that the transverse folds were formed when the different structural zones were rectilinear or weakly curved (Figs 2 and 3).

The Dent Blanche Nappe including high pressure granulite facies rocks generally considered to have been formed in the lower crust, belongs to the Insubrian Plate. This plate would have moved initially northwards upon the Piemontese zone, giving rise to high pressure-low temperature metamorphic conditions in the Piemontese units, before its westward later translation. Therefore the basal contact may be regarded as part of a subhorizontal palaeosubduction at the base of the Insubrian sialic crust.

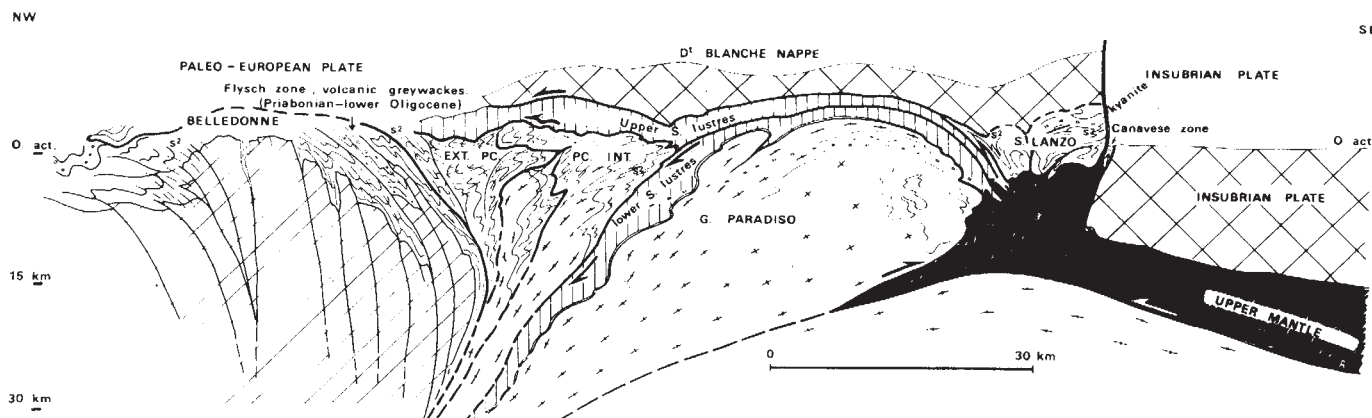
The major thrust, generally interpreted as a retrochevauchement, marking the eastern limit of the Briançonnais zone with external Carboniferous basement, may be regarded as an early major subduction zone, along which the movement revealed by the transverse plunging folds, was essentially horizontal (sinistral wrench fault dipping west). This zone of subduction has been later reactivated and tilted during the F_2 deformation, with a vertical post-metamorphic movement which is particularly spectacular in the Cottian Alps. We recall that the andesitic greywackes of Tavayanne³¹ (Priabonian to Lower Oligocene) may be the remnants of a volcanic arc in external position, possibly in connection with westwardly dipping subduction zones. It is not intended here to discuss early kinematics related to downgoing of structural zones and microplates toward the external part of the Alps, which could have acted in the first stages without any penetrative deformation. This subduction was possibly initially related to the expansion of a Tethyan ocean from a Piemontese ridge during Jurassic-Lower Cretaceous times⁸.

The constancy of transverse folds in the different structural zones implies horizontal movements and translations of low-angle thrust sheets and small plates of unknown extent towards the NNE, before the arc curvature. At this time (post middle Eocene), the Western Alps must have been a synthetic thrust belt³². It is significant that the deformation path compatible with transverse folds is not a contradiction with the motion model in the alpine system at this time deduced from magnetic anomalies in the Atlantic Ocean³³.

The Alpine arc has been generated during the second phase of Alpine deformation, which occurred after a complete change in the relative motion of plates³³. The arc curvature, formed by the collision of the Insubrian Plate moving westwards (300 km according to Laubscher), reflects the shape of its western edge. The contraction which resulted (Fig. 4), would have been absorbed by three main mechanisms: (1) deep outward under thrusting in the lower crust³⁵ inducing areas rapidly uplifted and eroded; (2) contraction of each zone by F_2 folding (outward and inward overturned folds are synchronous) mostly responsible for a post-metamorphic flexuration of S_1 cleavage and foliation with sub-vertical Z and for isoclinal folding in the more ductile zones during intense tangential movements. The amount of shortening gradually decreases toward the external zones, where the F_2 folds and cleavage are also younger (post Oligocene) and followed by post Miocene folds, than those of the inner zones (Lower Oligocene); (3) large scale outward translations of the higher units on a continuously rejuvenated and outward moving slope.

This late Alpine phase is characteristic of an antithetic thrust belt³². The second phase of Alpine deformation is also responsible for the rapid uplift of high pressure-low

Fig. 4 Transverse schematic section of the western Alps (partly after J. G. Ramsay for the External zone, Ellenberger¹¹ for the Permo-Carboniferous and Giese³⁵ for the inferred structure of the Ivrea zone).



temperature metamorphic zones before the establishment of a normal geothermal gradient^{7,28} which would have destroyed the metamorphic assemblages as in the central Alps. A considerable part of the global shortening would also have been absorbed by subduction of the schistes lustrés and internal crystalline basements under the Briançonnais zone and external carboniferous basement.

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Triassic seaways and the Jurassic Tethys Ocean in the central Mediterranean area

In the central Mediterranean area phases of middle Triassic and middle Liassic rifting are recognisable. The former produced only relatively deep pelagic basins founded on thinned continental crust; the latter, however, marked the opening of the central Tethys. The Porphyrit-Hornstein and the Diabas-Hornstein formations¹ are the results of such phenomena. During Dogger and Malm times the spreading continued and a large amount of oceanic crust was formed. The Tethys Ocean opened parallel to the long axes of some Triassic deep sea basins and across the long axes of others. Consequently, the positions of Triassic seaways along the southern continental margin of the Tethys 'geosyncline' are defined by the effects of at least two main palaeotectonic events.

In the Triassic System the Germano-Andalusian facies is characterised by prevalent continental deposits, whereas the Alpine facies is characterised by marine deposits. In the circum-Mediterranean area the basements of both Germano-Andalusian and Alpine facies sediments everywhere comprise continental crust which may underlie Palaeozoic sediments and volcanics. Only in the Antalya Nappe (Turkey) are there indications that a crust of oceanic composition underlies Alpine facies sediments.

The Alpine facies, occurring mainly in the Upper Triassic, is generally represented by shallow water carbonates (dolomites and limestones), and in certain areas by evaporites. Locally,

Halobia limestones are known, showing relatively deep sea environments. The outcrops of *Halobia* limestones form narrow belts, more or less disconnected, which stretch along the circum-Adriatic chains from Greece to Sicily.

The complete Triassic sequence containing the *Halobia* limestones is not much varied in the whole Mediterranean region. The Lower Triassic is represented by shallow water, terrigenous sediments and acid volcanics, which are a prolongation in time of the Upper Permian facies. The Middle Triassic is represented by terrigenous sediments simulating flysch deposits (Monte Facito Formation in the Apennines and Sicily; Montenegro 'flysch' in Yugoslavia), by mafic volcanics (Porphyrit-Hornstein Formation in Yugoslavia and in Greece), and less frequently by nodular limestones like Ammonitico Rossa (Han Bulog Beds in Yugoslavia and in Greece). The Upper Triassic is represented by *Halobia* limestones with interbedded rare mafic lavas and hyaloclastites, and locally by nodular limestones like Ammonitico Rosso (Hallstatt Beds in the eastern Alps).

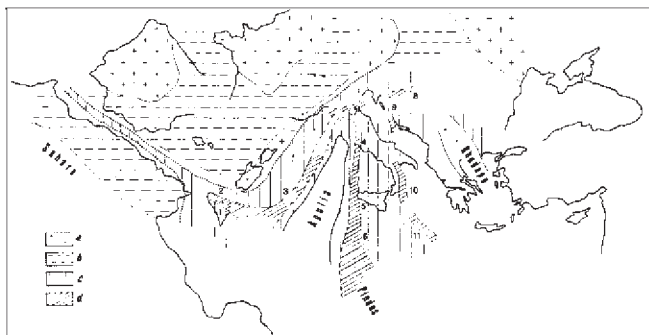
The *Halobia* limestones have been considered as markers of an oceanic Palaeo-Tethys in the central Mediterranean area^{2,3}. I suggest, however, the basins to which pelagic pelecypods and ammonites migrated originated from an eastern oceanic Palaeo-Tethys and penetrated deeply into a continental Palaeo-Mediterranean gulf as 'seaways' founded on thinned continental crust. Among these Triassic seaways the most considerable is the Pindos Basin, which stretches for more than 1,200 km through Greece, Albania and Yugoslavia. On the opposite side of the Adriatic Sea, after a gap of some 100 km, the basin is recognisable in the southern Apennines-Sicily arc as unconnected outcrops stretching for about 800 km.

In Jurassic times a real ocean separated the European and the African continents. Contemporaneously, large shearing movements began between Europe and Africa as a result of the opening of the southern Atlantic⁴.

Jurassic Tethyan deposits consist of mafic and ultramafic rocks, radiolarites, pelagic limestones, claystones and sandstones, frequently affected by high pressure-low temperature metamorphism.

A basic assumption in any attempt at a palinspastic restoration of Triassic and Jurassic palaeogeography^{5,6} is that the volume of continental crust remained roughly the same before and after the Alpine deformation. Consequently, the kinematic system is considered a closed system. But a lot of continental crust must have disappeared into the asthenosphere^{6,7} and, therefore, huge volumes (or surfaces in a two-dimensional

Fig. 1 Palinspastic restoration of Upper Triassic palaeogeography in the central Mediterranean area. a, Stable areas with slight subsidence; b, subsided basins of Germano-Andalusian facies; c, shallow-water carbonates of Alpine facies; d, 'seaways'; 1, Sicani zone; 2, Imerese zone; 3, Lagonegro zone; 4, Triassic pelagic sediments of Slovenia; 5, Budva-Kotor zone; 6, Pindos zone; 7, Australpine of Hallstatt; 8, Australpine of the Gomerides; 9, Australpine of the Balaton Lake; 10, Subpelagonian Triassic pelagic limestones of Serbia; 11, Subpelagonian Triassic pelagic limestones of Northern Pindos and Othrys.



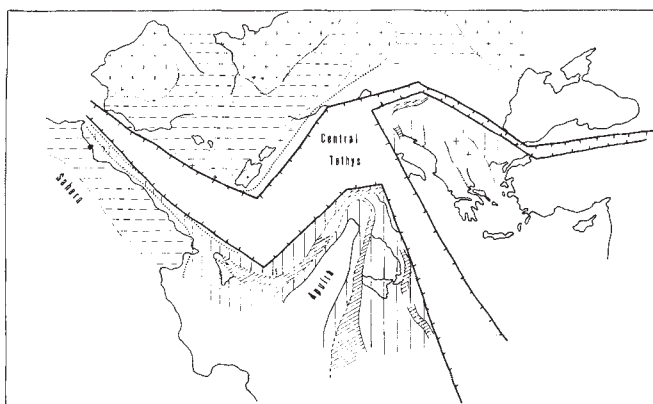


Fig. 2 The Tethys Ocean in the central Mediterranean area.

calculation) have been taken out of the system. These lost volumes are not counterbalanced by additional small volumes of new oceanic crust. In any palinspastic restoration it is therefore necessary to smooth out all the nappes of the peri-Mediterranean Alpine systems to calculate the real shortening of the plate margins during the Alpine compression. Figures 1 and 2 attempt such a palinspastic restoration for the Upper Triassic and Upper Jurassic. The original position of the palaeogeographical units has been obtained by fixing the 'stable' European and the Saharan plates, smoothing out folds and nappes, removing the main lateral displacements and connecting those belts which are common to the African and European margins. This construction of the Western part of Fig. 2 relied upon the palinspastic restoration of Laubscher⁷.

This reconstruction produces an area corresponding to the Alpine facies, forming a gulf open in the direction of the Palaeo-Tethys and surrounded by regions characterised by Germano-Andalusian facies. In these regions it is possible to distinguish stable zones, slightly subsident and unaffected by synsedimentary tectonics, and less stable or clearly unstable zones, characterised by high rates of subsidence and sedimentation, synsedimentary faulting and mafic volcanism.

In the area comprising Alpine facies the palaeogeography changes radically from the Lower to the Upper Triassic because of a Middle Triassic tectonic phase, which was responsible for the tectonic regime immediately before the opening of the Tethys. This tectonic phase included an attempt at rifting, which produced a complex system of tensional faults that crossed the Palaeo-Mediterranean Gulf in several directions. The faulted blocks underwent different vertical movements. The uplifted blocks often underwent severe erosion, whereas flysch-like sediments were deposited on the downshifted blocks. Contemporaneously, widespread mafic magmatic activity (comprising an alkali-basaltic series) occurred along the main faults. Deep sea basins developed along preferential directions (probably corresponding to strips of crustal thinning) within the Palaeo-Mediterranean Gulf, allowing the diffusion of the pelagic fauna from the Triassic Palaeo-Tethys through Turkey, Greece, Albania, Yugoslavia, Hungary, Czechoslovakia, Austria, Italy, and as far as western Sicily. The absolute homogeneity of the fauna proves that close intercommunications were established between all of the seaways. Figure 1 shows the region during the late Triassic.

During Liassic times, starting mainly in the Middle Liassic, important new events occurred in the central Mediterranean area: along giant tensional fractures, partly parallel to, and partly discordant with, the former seaways, the Tethys Ocean began to open. In the internal nappes of the Dinarides and of the Hellenides at least two main ophiolite assemblages deriving from the oceanic Tethys may be distinguished: the Diabas-Hornstein Formation, and the ultrabasic massifs at Zlatibor and Vourinos, with their basalts and sediments. The

Diabas-Hornstein Formation comprises alternated diabbases, radiolarian cherts, sandstones, claystones, graded calcareous microbreccias, allodapic and pelagic limestones, and intra-formational conglomerates. This sequence may overlie Jurassic limestones founded on continental crust⁸.

The generally tectonic basal contact and the occurrence of slices of serpentinites between the Jurassic limestones and the Diabas-Hornstein Formation suggest that the latter (at least in part) was possibly founded on oceanic crust. On the other hand, the occurrence of terrigenous material and of neritic calcareous turbidites suggests provenance on a very near continental platform. I suggest that the Diabas-Hornstein Formation is the product of the Liassic rifting which marked the beginning of the opening of the Tethys Ocean, so it may be considered as a marker of the continental margin or the continental rise. As the opening of the Tethys Ocean continued, the areas in which the Diabas-Hornstein Formation had been deposited were shifted laterally and, according to plate accretion models, new areas of oceanic crust were generated in their place. These new areas of oceanic ridges which are, of course, younger than the Diabas-Hornstein Formation, are today recognisable as the ultramafic massifs.

In the Alps and in the Apennines this distinction between initial rifting (Diabas-Hornstein Formation) and real ocean spreading (ultramafic massifs) of the central Tethys is not so clear as it is in the Dinarides, but it is probable that the Ligurian and Piemontese ultramafic rocks represent an ancient, oceanic ridge area, whereas parts of the Southpennine *Bundnerschiefer* represent an equivalent of the Diabas-Hornstein Formation.

During the Middle and Upper Liassic the combination between the Atlantic and the Tethyan movements produced a renewal of synsedimentary tectonic activity, and consequently the palaeogeography became modified not only around the central Tethys, but also around the former Triassic seaways. For example, in western Sicily the former Imerese seaway, which during the Upper Triassic ended at the longitude of Palermo, suddenly lengthened westwards during the Liassic, in the direction of the Maghreb along a newly generated deep furrow in which radiolarian oozes and calcareous turbidites stratigraphically overlie collapsed shallow water dolomites⁹. Contemporaneously, in areas of maximal tension (Imerese, Sicani, Pindos), flows of alkali-basalts were erupted.

During post-Jurassic times the Triassic seaways near to the margin of the Tethys Ocean, and those which were cut across by the opening of the ocean, were affected by Cretaceous compression. These orogenic phases, the earliest in the central Mediterranean area, were responsible for the reduction, and the local disappearance, of large oceanic zones. In Greece (Northern Pindos¹⁰ and Othrys), sediments deposited very near to the Tethys Ocean-Triassic seaways were affected by Cretaceous compression, and were involved in the building of the ophiolite nappes.

On the other hand, the continuing sedimentary history of those seaways which lie more within continental areas, will cease only after the collision between Europe and Africa. In Sicily (Sicani Mountains¹¹), such sedimentary deposits were affected by compression during the Upper Miocene, when Europe and Africa had already collided and a lot of continental crust from both plates had disappeared because of subduction.

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Laser microprobe mass analysis of organic materials

LASER microprobes currently in use combine a laser microscope with a light emission spectrometer¹⁻³. Recent improvements in instrumentation have demonstrated the limitations of this method with respect to spatial resolution (some μm) and detection capability (10^{-13} - 10^{-15} g). Much lower detection limits (10^{-18} g) have been obtained in ultrathin sections with electron probe X-ray microanalysers^{4,5}, although the latter, however, do not satisfy many needs of biologists. Neither trace

kinetics of Na^+ , K^+ , Mg^{2+} , and Ca^{2+} , indicating a need for microprobe techniques capable of analysing isotopes. A mass spectrometer in combination with a laser microprobe seems to be a very attractive approach. In principle, it should enable not only high sensitivity but also isotope analysis and the 'fingerprinting' of organic molecules, provided they do not decompose into fragments too small for recognition of the parent molecules. The special requirements for biomedical application are satisfied by the laser microprobe mass analyser (LAMMA) described here.

The instrument (Fig. 1) combines a time-of-flight mass spectrometer with a laser optical device. The latter consists of an incident light microscope and a Q-switched, frequency-doubled ruby laser ($\lambda=347$ nm). The specimen is mounted on a formvar-coated electron-microscope grid fixed to the lower side of a microscopic cover glass with no further support underneath (Fig. 1). In addition the cover glass serves as the vacuum window of the time-of-flight mass spectrometer. This arrangement enables free expansion of the laser-generated microplasma into the mass spectrometer. Maximum optical resolution is obtained using an immersion objective. Sampling areas (down to $0.5 \mu\text{m}$ in diameter and almost limited by diffraction) could be achieved reproducibly in a rather broad variety of unstained biological materials by adjusting the laser power density in the focus between 5×10^8 and $5 \times 10^9 \text{ W cm}^{-2}$ (ref. 6).

The time-of-flight spectrometer consists of a 3 kV acceleration grid, 4 mm below the sample plane, an einzel lens, a field-free drift tube of approximately 1 m in length and an electron multiplier as ion detector. The overall transmission of the mass spectrometer depends on the directional distribution of the initial ion velocity. Rough calculations indicated that a transmission of 1% or better could be achieved if the initial energy of the ions remained below 50 eV. Measurements showed that, in the conditions chosen, ions had mean initial energies of 5-30 eV (ref. 6). Accordingly the calculated peak half width at mass number 39 is less than 0.15 a.m.u. which means that, theoretically, a resolution of $m/\Delta m=260$ should be possible. The experimental peak half-width is, however, limited by the recording electronics, which consisted of an impedance matching preamplifier, a fast transient recorder and a strip chart or magnetic tape recorder. To determine sensitivity and resolution of the microanalyser as well as the reproducibility of recorded spectra, standard specimens were prepared according to a suggestion made by Spurr⁷. Organometallic complexes formed from macrocyclic polyethers and inorganic ions such as Li^+ , Na^+ , Mg^{2+} , K^+ , Ca^{2+} and Co^{2+} were dissolved in epoxy resin to give final doping concentrations in the range 10^{-2} - 10^{-6} weight fraction. Sections (0.1 - $1.0 \mu\text{m}$ thick) were cut on a microtome.

Figure 2 shows two mass spectra obtained from such epoxy standards doped with Li, Mg, and Co. In spectrum (a) the expected signals of Li, Mg, and Co can be readily identified as well as those from Na and K contained in the epoxy resin as impurities. Most of the other peaks seem to represent fragments of the organic matrix. In spectrum (b) many of these signals exceed the dynamic range of the transient recorder, which was set to obtain optimum recording of the

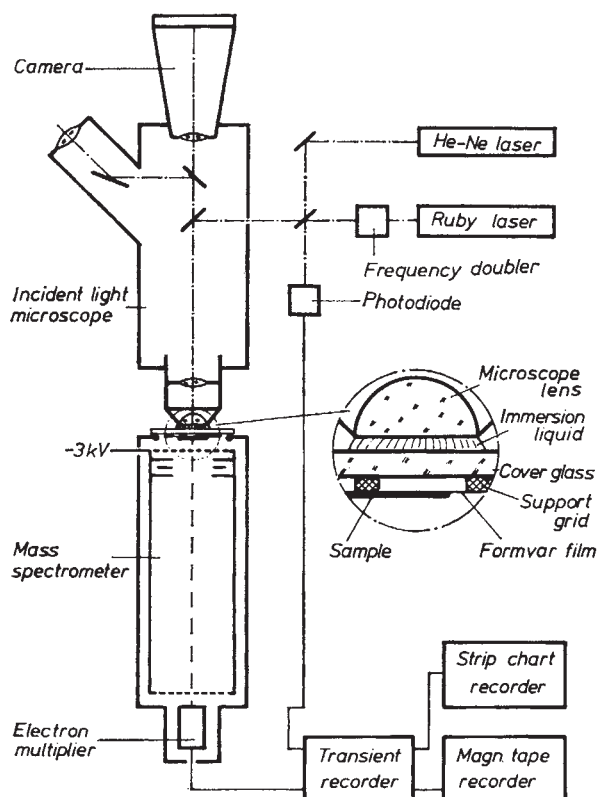


Fig. 1 Schematic diagram of laser microprobe mass analyser.

element detection in the parts per 10^9 range nor analytical information about organic constituents can be obtained. In the case of the main physiological electrolytes, biologists are primarily interested in the cellular and subcellular transport

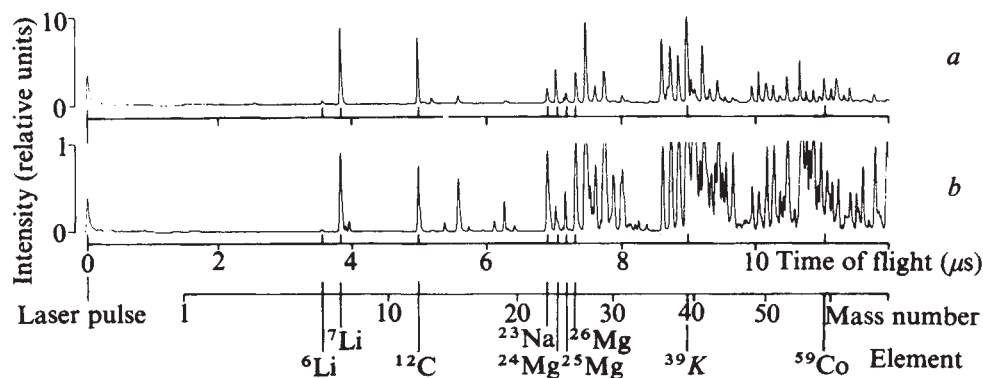


Fig. 2 Mass spectra obtained from epoxy resin, doped with Li, Mg, and Co. a, Sample doped with 4×10^{-5} weight fraction (40 p.p.m.) of each of the inorganic ions. b, Sample with a doping of only 5×10^{-6} (5 p.p.m.). b was recorded at an instrument sensitivity ten times that of a. In both cases the sampled volume was $3.5 \times 10^{-13} \text{ cm}^3$ corresponding to a mass of $3.9 \times 10^{-13} \text{ g}$ (diameter $1.5 \mu\text{m}$, thickness $0.2 \mu\text{m}$). The electronically added laser signal serves as zero time mark of the time-of-flight scale and as laser output control.

Li isotopes. The relative peak heights of ^6Li and ^7Li correspond quite reproducibly with the natural abundances of 7.5% ^6Li and 92.5% ^7Li . For Mg this correspondence is lost in the statistical fluctuations of the rather small signals at mass numbers 24 (natural abundance 79%), 25 (10%) and 26 (11%). A partial masking of the Mg signals by mass interfering molecules may be one of the reasons for this.

The overall sensitivity of the instrument is estimated from spectrum (b). With a probed mass of 3.9×10^{-13} g and ^6Li present at a weight fraction of 4×10^{-7} (0.4 p.p.m.), 1.4×10^{-19} g or 1.4×10^{-4} atoms ^6Li were detected. This sensitivity is at least one order of magnitude better than that achieved in electron probe X-ray microanalysis. Further improvements are possible as in this experiment neither the multiplier nor the preamplifier were optimised.

Peak half-widths so far obtained are less than 0.3 a.m.u. at mass number 39 (K). This is, of course, more than is needed to resolve natural isotopes. In mass ranges in which large amounts of organic fragments occur, however, it may be difficult to separate small peaks of trace elements from those of components with nominally the same mass number, for example, Si may be obscured by C_2H_4 or CO. On the other hand, preliminary spectra from freeze-dried specimens of kidney, frog skin, or human erythrocytes have shown Na and K peaks much in excess of those of the organic fragments, the ion yield of which is obviously comparatively small.

In spite of the positive features of the method with respect to sensitivity and resolving power, quantitative analysis still poses some problems. Both ion yield and transmission depend on the ion species under investigation. Moreover the laser radiation-solid interaction involves some highly non-linear processes which make reproducibility from shot to shot rather poor. The best results so far achieved with standard specimens exhibit a s.d. of $\pm 20\%$ in the case of ^7Li . The signals of other elements vary by more than an order of magnitude (C peaks, Fig. 2). This is approximately twice the s.d. found in electron probe X-ray microanalysis of similar specimens⁸. It is expected that systematic optimisation of the laser operating conditions will overcome this problem.

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Stability of complex ecosystems

ECOLOGISTS have generally held that a complex ecosystem is more likely to be stable than is a simpler one. Gardner and Ashby¹ have shown, however, that for randomly connected linear systems the probability that the equilibrium will be stable actually decreases as the complexity increases. Of course, whether or not this is a contradiction depends very much on what is meant by stability. It is doubtful that field ecologists are really thinking of the relative likelihoods that systems of

different types will persist; it is, after all, only those systems which persist which are observed and about which generalisations are made. The reason for connecting stability and complexity seems rather to have been the observation that large scale population oscillations are much more common in simple ecosystems than in complex ones^{2,3}. We have therefore considered the following problem: Given that a system is near a stable steady state, will increased complexity tend to increase or to decrease the rate at which perturbations die out?

We have constructed random matrices according to the procedure suggested by Daniels and Mackay⁴: the diagonal elements a_{ii} ($i=1, 2, \dots, N$) were taken from the uniform distribution on $(-1, -0.1)$ and a specified fraction C of the off-diagonal elements were taken from the uniform distribution on $(-1, 1)$. The remaining elements were set equal to zero. We found the eigenvalues λ_j , discarded all those matrices which were not stable, and for the stable matrices computed the value of

$$D = \frac{1}{N} \sum_{j=1}^N \exp\{\text{Re}(\lambda_j)\}$$

This represents the magnitude after one unit of time of a unit perturbation which was originally distributed equally in all the eigendirections, and is thus a measure of the mean damping in the associated linear system. We also computed D averaging over all matrices, stable or not, but, as we expected, D then increased with both N and C : for $N=8$ and $C=0.6$, for example, D was 0.721.

The observed lack of dependence of D on complexity may be surprising, but there is evidence to show that it is not artefactual. For example, the similar expression $\exp(\sum \text{Re}(\lambda_j)/N)$, which is formed from the trace of the stability matrix, is totally independent of both N and C , and although it is not in general equal to D , the difference is much smaller when all the $\text{Re}(\lambda_j)$ are negative, as the effect of exponentiation is less. Consider also the two-species linear system, the eigenvalues for which are

$$\lambda = \frac{1}{2}[a_{11} + a_{22} \pm ((a_{11} - a_{22})^2 - 4a_{12}a_{21})^{1/2}]$$

If the eigenvalues are complex, that is, if there are any oscillations at all, then the rate of damping is entirely independent of the interaction terms a_{12} and a_{21} . If, on the other hand, the eigenvalues are real, then if the system is to be stable the eigenvalues are restricted to the range $(a_{11} + a_{22}, 0)$. If a_{12} and a_{21} are larger than a_{11} and a_{22} , then the effect will not be to increase (or decrease) the damping, but either to produce oscillations or destabilise the system, depending on the sign of $a_{12}a_{21}$. These features are consistent with those found numerically in larger systems.

We conclude that whereas increasing N or C decreases the probability that a randomly connected linear system will be stable, once it is given that such a system is stable, the rate at which perturbations die out does not depend on the complexity. Damping seems to be caused mainly by the self-limitation terms, whereas the chief effect of interactions between species is to produce oscillations.

How can we reconcile these results with the existence in nature of complex ecosystems which are stable and in which there do not seem to be population oscillations on the same scale found in simpler systems? May⁵ has argued that we need not be unduly concerned about the prediction that complex systems are likely to be unstable, because what we observe is not a random sample of arbitrary systems but rather only those which have evolved in such a way as to produce stability and therefore persistence. But this line of reasoning does not explain the lack of oscillations in complex systems. We believe that the solution to both problems may lie in a difficulty in the definition of complexity first pointed out by Somoraji and Goswami⁶. The stability matrix is a matrix of first derivatives, and C (which ought properly to be called the linear connectivity) thus measures not the total number of inter-

Table 1 Persistence of oscillations as a function of complexity

N	C=0			C=0.3			C=0.6			C=0.9		
	σ	μ	n	σ	μ	n	σ	μ	n	σ	μ	n
4	0.08	0.594	0.08	437	0.596	0.08	302	0.596	0.07	217		
5	0.07	0.590	0.07	391	0.606	0.06	240	0.596	0.07	134		
6	0.06	0.589	0.06	354	0.600	0.06	138	0.608	0.05	70		
7	0.06	0.594	0.06	292	0.600	0.05	83	0.616	0.05	35		
8	0.05	0.594	0.06	194	0.596	0.05	46	0.593	0.04	19		
9	0.05	0.598	0.05	149	0.601	0.05	27	0.596	0.04	6		
10	0.05	0.585	0.04	85	0.621	0.03	9	—	—	0		
11	0.05	0.603	0.03	56	0.569	0.01	2	—	—	0		
12	0.04	0.599	0.04	27	—	—	0	—	—	0		

Mean (μ) and s.d. (σ) of D for values of N and C chosen to cover the range for which the probability of stability is non-negligible. Number of stable matrices (n) out of 500 in each case is also shown. When $C=0$ then $\mu=0.597$ for any N .

actions between species, but only those such that at equilibrium a small change in the population density of one species produces a direct change in the net specific growth rate of another. There seems no reason to suppose that the majority of interactions within an ecosystem fall into this class; it excludes, for example, the obviously vital connection between a predator and its only prey, in any situation in which the prey is in excess and the predator is limited by some other factor, such as space. If large, complex systems have the property that their linear connectivity is low, then they are more likely to be stable, and if stable, are more likely to be over-damped than to oscillate. An example of this behaviour can be found in the analysis of an n -species food chain⁷. And only ecosystems with relatively few species can have a sufficiently high linear connectivity to produce oscillations without becoming unstable—which would explain why it is that population oscillations are observed in the arctic but not in the tropics.

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Plant growth substances and effects of photoperiod on flower bud development in *Phaseolus vulgaris*

THE flower buds of certain varieties of *Phaseolus vulgaris* abscise in British summers, a phenomenon which is induced when the plants are grown in day lengths longer than 13–14 h¹. The number of buds which abscise increases as the day length is extended^{2,3}. This post-initiation effect is truly photoperiodic, the photoperiodic stimulus being received in the leaves and the inhibitory effects expressed in the buds, the growth of which is inhibited before they finally drop^{2–5}. Experiments in which a single trifoliate leaf was exposed to a photoperiod different from the rest of the plant provided indirect evidence that the effects of photoperiod on flower bud development were mediated through the production of substances in the leaves, with a predominance of inhibitor(s) being formed in long days and of promoter(s) in short days^{3,4}. We describe here the determination in a single sensitive variety, classified as P47, of the chemical basis for

the inhibitory and promotory effects of long and short days respectively.

Exogenous application of either indolylacetic acid or gibberellic acid to the leaves or flower buds of plants kept in short days failed to induce the bud abscission experienced in long days^{3,4}. In contrast, however, it has now been found⁶ that application of synthetic abscisic acid (ABA) to the differentiating flower buds of plants in short days results in inhibition and finally abscission of many of the buds at a later stage of development. For example, when ABA was applied in a droplet of solution daily for 7 d (range 0.1–5.4 μ g per application), the extent of flower bud abscission over the whole plant depended on the amount of ABA applied, the effect being most marked on those buds carried on the terminal inflorescence and at the uppermost node. Endogenous ABA extracted from plants grown in long days and re-applied to plants in short days evoked effects similar to those of the synthetic compound. On the other hand, exogenous application of the chemically related inhibitor, xanthoxin, did not induce flower bud abscission.

In view of the above results, indicating the possible participation of ABA in the regulation of flower bud development in this variety, numerous experiments were carried out during the summers of 1970–72 to study the effects of photoperiod on the endogenous concentrations of ABA. ABA, both free and bound, was extracted⁷ from different organs and the concentrations estimated, mostly by wheat coleoptile bioassay⁸, with some confirmed by gas liquid chromatography⁹. The two methods gave results of the same order whether the tissues contained low or relatively high concentrations of ABA; for example, two different samples of leaves gave results by bioassay and gas liquid chromatography, respectively, of 33.5 and 35.4 μ g per kg fresh tissue, and 225 and 208 μ g per kg fresh tissue, these figures all being averages of several complete analyses of each extract.

Our experiments showed that the leaves and buds of plants grown in long days contained more ABA than those in short days (Table 1). The concentrations of ABA in the leaves, buds, petioles and stems of plants grown in an 18 h photoperiod (in which the buds were severely inhibited) were higher than those in the corresponding organs of plants grown in an 8 h photoperiod (in which bud development was normal). These differences in ABA concentrations were large in the youngest leaves but smaller in the mature leaves. The highest concentrations of ABA were found in the inhibited buds; these buds also contained considerable amounts of bound ABA.

Table 1 Concentration of ABA (μ g per kg fresh tissue) in *Phaseolus vulgaris*, var. P47, grown in different photoperiods

Organ	Photoperiod	
	8 h	18 h
First trifoliate leaves	42	51
Second trifoliate leaves	21	37
Third trifoliate leaves	39	55
Fourth trifoliate leaves	30	91
Fifth trifoliate leaves (unfolding)	20	305
Combined petioles	11	36
Stems	18	29
Terminal flower buds	121 (9)*	729 (60)*

* Bound ABA in parentheses.

Seeds were sown on May 5, 1972. In any 24 h periods all plants received 8 h natural daylight in a greenhouse, followed by either 0 or 10 h incandescent light (540 lx at pot level) in two photoperiod rooms, each maintained at $20 \pm 1^\circ\text{C}$. Plants were collected on June 7 when the final trifoliate leaf was unfolding and the flower buds on the plants in the 8 h photoperiod were just about to open. The individual organs from the plants were combined and extracted, and determinations were carried out by wheat coleoptile bioassay.

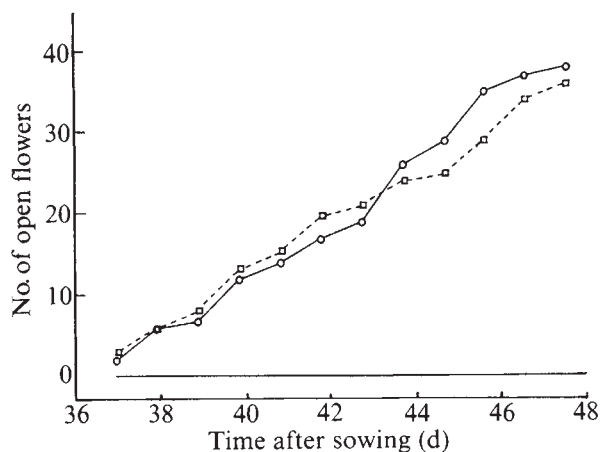


Fig. 1 Effect of cytokinins on the cumulative number of open flowers in long days. Plants were grown in a greenhouse for 8 h, extended to 16 h by incandescent light in a photoperiod room. From 15 d after sowing 10 μ l solution containing 0.1 μ g kinetin or 6-benzyl-aminopurine were added to the stem apex daily for 10 d. Data are totals of five plants. ○, Kinetin; □, 6-benzyl-aminopurine. Control values remained at zero.

The above results, together with the effects of exogenously applied ABA, provide strong evidence that ABA plays an important role in mediating the effects of long days on flower bud development in variety P47. It would seem that under the influence of long photoperiods there is a greater production of ABA in the leaves and an increased accumulation of the substance in the buds, leading eventually to their inhibition and abscission. This was substantiated by a single experiment in which the abscising buds were found to contain more free and bound ABA (331 and 70 μ g per 100 g dry weight) than they did 6 d previously (105 and 0 μ g per 100 g dry weight, respectively).

Evidence that promotory as well as inhibitory substances are involved in mediating the effects of photoperiod was obtained when cytokinins were applied to bean plants grown in long days¹⁰. Here it was found that kinetin or 6-benzyl-aminopurine stimulated the development of flower buds which would otherwise have dropped (Fig. 1). No study has yet been made on the endogenous concentrations of cytokinins in the bean variety P47; note, however, that the concentrations of cytokinins in both the leaves of *Begonia*¹¹ and the xylem sap of *Perilla*¹² were found to be higher in short days than in long days.

Previous work on the inhibitory and promotory effects of photoperiod on flowering in various species has concentrated on the process of floral initiation or more generally on flowering, and the identification of the plant growth substances involved has proved difficult¹³. The present study is therefore of particular significance in referring specifically to the effects of photoperiod on flower bud development after the initiation of the floral primordia, and provides evidence as to the identity of the inhibitory and promotory substances involved.

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Suppression of apparent movement during binocular rivalry

STEREOSCOPIC depth perception can coexist with binocular rivalry of form and colour^{1–3}. If a stereogram is viewed with a red filter in front of one eye and a green filter in front of the other eye (or with an oblique grating superimposed on one eye's picture and an orthogonal grating on the other picture), then stereopsis can still be obtained in spite of the two eyes' pictures being seen alternately rather than simultaneously. Although one image is 'suppressed' in consciousness, information about its position is preserved and used for stereopsis. This finding implies either that stereoscopic depth perception 'precedes' the site of binocular rivalry in the visual system or that conscious perception and stereopsis are independent visual functions mediated in 'parallel' neural channels. Here I consider the related question of whether an image that is suppressed during binocular rivalry (and is, therefore, not 'seen' by the observer) can contribute to the perception of apparent movement.

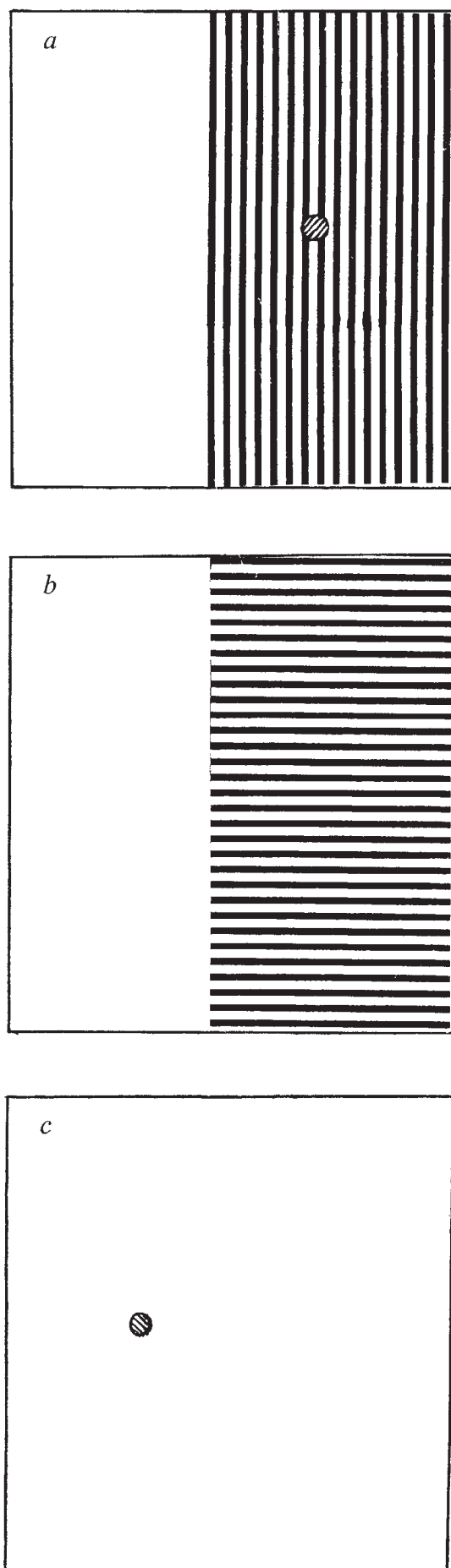
The display used in the experiment is illustrated in Fig. 1. Figure 1a and b were presented to the right and left eyes, respectively, in a three-channel tachistoscope driven by an electronic timer. They subtended 9° and were of equal average luminance. Crossed polaroids were used to separate the two eyes' pictures. The stimuli were high contrast horizontal (Fig. 1b) and vertical (Fig. 1a) gratings presented to one half-field alone in each eye. Figure 1a contained, in addition, a bright round yellow spot fixed to the centre of the grating. Figure 1c was displayed on the third channel either to the left eye alone (in one experimental condition) or to both eyes (in another condition), and contained only a yellow spot.

Four experienced observers were used in the experiment. They were instructed to press a button which illuminated Fig. 1a and b simultaneously. These would stay on as long as the button was held pressed. When the button was released a and b would go off simultaneously and c would appear in the other half-field after a brief delay of 75 ms.

Initially b was excluded from the circuit and the observers familiarised themselves with apparent movement of the yellow spot between a and c by alternately pressing and releasing the button (the distance between these spots was about 3.5°). b was then included in the cycle. When the observers now pressed the button they could see rivalry between the striped half-fields in a and b. Sometimes periods of complete suppression would be observed and no yellow spot would be seen when the horizontal grating in the left eye b was suppressing the vertical grating in the right eye a. The observers were instructed to release the button during such periods of complete suppression of a and to observe whether the yellow spot in c seemed just to appear from nowhere or whether it seemed to move to its present location from its original (imaginary) location in a. They were encouraged to repeat the procedure as often as they liked before making a decision. Specifically, they were asked to compare the effect of releasing the button during suppression of a, when the yellow spot was not visible, with the effect of releasing it during suppression of b.

All four observers reported that no apparent movement

Fig. 1 a, Right; b, left; c, left or both.



could be seen if the button was released when the yellow spot was being suppressed, suggesting that retinal rivalry precedes movement perception in the visual system. This was true irrespective of whether *c* was presented to the left eye alone or to both eyes. One of them, however, noted that if the button was released immediately following suppression then some vague impression of movement could, in fact, be obtained, presumably because the suppression itself took the place of switching off the light spot. If there was any delay between suppression and button release, then, like the other three observers, he saw no movement but only a single light spot *c* blinking on and off.

In this context it may be useful to think of apparent movement as an 'unconscious inference'⁵⁻⁷ that a visible object has changed its location rather than as an expression of the time constants of hypothetical 'motion detectors'. The brain may judge it improbable that the disappearance of a stimulus should be followed so soon by the sudden and independent appearance of an identical stimulus nearby and may therefore decide that the original stimulus must in fact have moved to this new location. As conscious perception may be a necessary prerequisite for any cognitive inferential process of this nature it is perhaps not surprising that a suppressed and invisible image cannot contribute to apparent movement perception.

This view is not necessarily inconsistent with the ideas of Anstis⁸, Julesz⁹ and Braddick¹⁰, as apparent movement is probably not a unitary process. The effects observed by these investigators using Julesz patterns may well be the result of activation of motion detectors at a relatively peripheral level in the nervous system. In fact Julesz⁹ and Kolers⁴ have argued before that motion perception may involve many different levels of processing.

The suppression of apparent movement during rivalry raises the related question of whether a single image can simultaneously contribute to stereopsis and apparent movement perception. To answer this question, the two half-images of a wheatstone stereogram were presented briefly to each eye alternately rather than simultaneously. (The interstimulus interval was 100 ms, and each stimulus lasted 100 ms.) If the disparity was large (1–2°) then the observers reported simultaneous stereo-depth and interocular horizontal apparent movement. This observation suggests that movement and depth perception are probably mediated in parallel neural channels with overlapping time constants.

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Immunobiology of membrane-bound collagen on mouse fibroblasts

THERE is ample evidence that collagen is produced by fibroblasts¹⁻⁵. Following biosynthesis in ribosomes, collagen is thought to be transported to the periphery by microtubules⁶ but very little is known about the biology of collagen in cell membranes. We report that collagen is membrane-bound, that it is patched and capped by anti-collagen sera, and that the

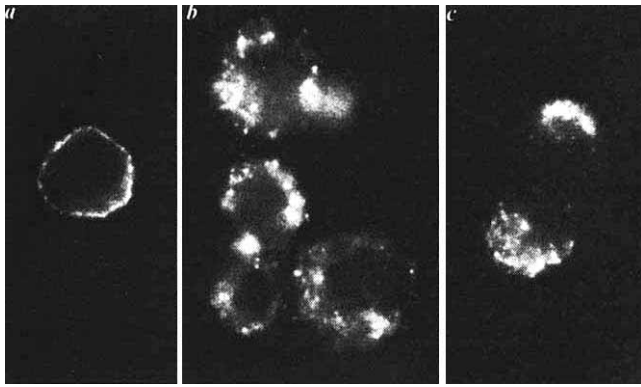


Fig. 1 Membrane immunofluorescence of mouse L-cell fibroblasts with rabbit anti-collagen serum. Reaction at 4°C (a), 22°C (b) and 37°C (c). Cells (1×10^6) in 1 ml Eagle's MEM containing 3% FCS and HEPES buffer at specified temperatures were reacted with 30 µl of rabbit anti-rat skin collagen sera for 20 min, washed twice in medium, stained with fluorescein isothiocyanate (FITC) sheep anti-rabbit Ig (Wellcome, UK) for 20 min, washed three times in medium, suspended in 40% glycerol, and examined for membrane immunofluorescence in a Zeiss photomicroscope 2 equipped for epi-illumination¹⁷. Anti-collagen specificity was indicated for the antiserum according to the following major criteria: (1) by showing that the amino acid content of the antigen was consistent with collagen, and that the antigen contained no anti-rat or anti-mouse serum proteins using immunoelectrophoresis and Ouchterlony analysis; (2) by showing that the antiserum stained rat and mouse tissues in a collagen distribution using immunofluorescence according to Rothbard and Watson¹⁶; that the antiserum agglutinated collagen-coated sheep erythrocytes treated with tannic acid and that the staining of tissues and fibroblasts as well as the agglutination of red cells was removed by chromatography of the antiserum on rat collagen-coated beads of CnBr-activated sepharose or Degalan beads (Degussa, Frankfurt); (3) by showing that the antiserum did not precipitate rat or mouse serum proteins by immunoelectrophoresis or Ouchterlony analysis, and that it did not stain the cell membranes of mouse leukocytes or erythrocytes; (4) by showing that the antibody stained fibres in mouse tissues with collagen periodicity in the electron microscope using the antibody-peroxidase technique²⁵.

mobility of membrane-bound collagen is influenced by temperature and vinca alkaloids. The identification of membrane-bound collagen as a fibroblast marker would augment existing definitions of these cells, and would perhaps allow the application of certain immunological techniques to study collagen secretion by manipulating membrane-bound collagen with antibodies, lymphocytes, and so on.

Several proteins and receptors on fibroblasts have been studied immunologically. Histocompatibility antigens of mouse fibroblasts patch and cap with anti-H2 sera^{7,8}; concanavalin A (con A) receptors on mouse 3T3 cells patch when treated with con A-ferritin complexes and anti-ferritin⁹; phytohaemagglutinin (PHA) receptors have been demonstrated on mouse L cells by agglutination and immunofluorescence techniques (A.T. and W.P.F., unpublished); and several types of mouse fibroblasts form spontaneous rosettes with erythrocytes^{10,11}. The redistribution of these membrane structures are generally inhibited by low temperature or certain chemicals, usually vinca alkaloids. Although these reactions are specific, they are not unique to fibroblasts.

We used mouse (H2^k) L-cell fibroblasts grown in Eagle's MEM supplemented with L-glutamine, 10% foetal calf serum (FCS) and 20 µg ml⁻¹ ascorbic acid. These cells were judged morphologically to be fibroblasts, and fixed cells were indirectly stained for collagen with anti-collagen serum¹². The fibroblasts were grown to confluence in Falcon 3025 tissue culture flasks (75 cm²) and removed using either 0.2% trypsin for 10 min or 1% EDTA in phosphate buffered saline (PBS). After washing, the cells were suspended at a concentration of 1×10^6 per ml in Eagle's MEM containing HEPES buffer and 3% FCS. Their

viability was greater than 99% as demonstrated by the exclusion of 0.1% trypan blue and by the intracellular hydrolysis of fluorescein diacetate¹³. These cells were then studied for membrane immunofluorescence using rabbit antisera to acid-soluble rat skin collagen. The antisera were prepared by first extracting an acid-soluble collagen antigen from rat skins with acetic acid according to the method of Bornstein and Piez¹⁴. The rat skin collagen, precipitated seven times, contained no rat serum proteins as demonstrated by immunoelectrophoresis against a potent, precipitating sheep antiserum to rat serum proteins, and the amino acid content of this antigen was consistent with collagen. Antisera were raised in rabbits immunised with 2 mg collagen in 1.5 ml Freund's adjuvant containing heat-killed *Mycobacterium tuberculosis*, followed 1 month later by 2 mg alum-precipitated¹⁵ collagen intraperitoneally. A 1:100,000 dilution of the antisera agglutinated collagen-coated tanned sheep red blood cells, and this was inhibited by the addition of native rat or mouse skin collagens but not by native human skin collagen. The antisera did not precipitate rat or mouse serum proteins by immunoelectrophoresis. The sera produced a collagen pattern of staining¹⁶ on cryostat sections of rat and mouse tissues but not on human tissues. The immunofluorescence in all of these experiments was carried out according to Faulk and Hijmans¹⁷ (see Figs 1 and 2).

Viable L-cell fibroblasts produced a patchy, occasionally capped, pattern of membrane spots when stained at 22°C with rabbit antisera to rat skin collagen (Fig. 1b). When this was performed at 4°C the pattern of staining was homogeneous (Fig. 1a), and capped cells were seen when it was carried out at 37°C (Fig. 1c). The transition from 4°C to 37°C (that is, transition of staining patterns from homogeneous to patch to cap) involved the coalescence of discrete membrane spots into a cap (Fig. 2). This transition did not occur in the presence of either colchicine or vinblastin, but was observed in the presence of cytochalasin B (Table 1). Cells treated at 37°C with colchicine or vinblastin did not cap but had a characteristic pattern of peripheral staining. We were unable to observe a

Fig. 2 Coalescence of membrane-bound patches into a cap of collagen staining. L-cell fibroblasts treated with rabbit anti-collagen sera and FITC sheep anti-rabbit Ig. Control reagents for this experiment included (a) normal rabbit serum, normal human serum and normal sheep serum; (b) sheep anti-human normal serum and sheep anti-con A, and (c) FITC sheep anti-swine Ig and FITC rabbit anti-human Ig. All of these were negative. Arrows indicate the general direction of movement for patches of membrane-bound collagen to form a cap. We anticipate that, under suitable conditions, this is followed by secretion of collagen¹¹.

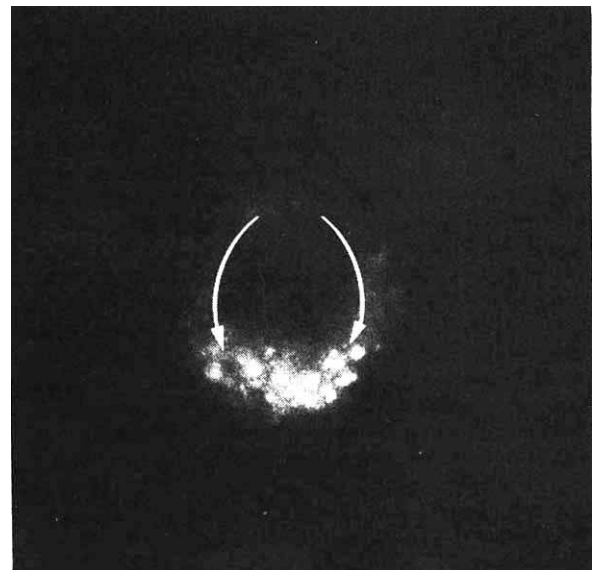


Table 1 Effect of temperature, cytochalasin B and vinca alkaloids on fibroblast patch and cap reactions with anti-collagen sera

Temperature	Reagent	Amount of reagent	Patch (%)	Cap (%)
4° C	PBS	—	5	none
37° C	PBS	—	>95	35
37° C	Cytochalasin	10 µg ml ⁻¹	>95	37
37° C	Colchicine	10 ⁻⁸ M	>95	<5
37° C	Vinblastin	10 ⁻⁸ M	>95	<5

Cytochalasin B (Aldrich, UK), colchicine and vinblastin (Sigma, UK) were used. The cytochalasin B was dissolved in dimethyl sulphoxide at a concentration of 10 mg ml⁻¹. DMSO control had no observable effect on membrane immunofluorescence.

difference in collagen staining between cells collected using trypsin and EDTA.

Incubation of trypsin harvested cells for 1 h with 50 units of collagenase (BDH) under optimal conditions for the enzyme¹ still gave the usual staining pattern with anti-collagen sera. Subsequent incubation of collagenase-treated cells with 500 units of pronase (Koch-Light, *S. griseus*) for 1 h at 37 °C became negative for collagen staining. Pronase alone, however, was found to be responsible for this result. Pronase-treated cells giving no reaction for membrane-bound collagen become positive after 3 h incubation in complete medium containing ascorbic acid. The failure of collagenase to remove membrane-bound collagen is consistent with the observation that rabbit anti-collagen serum is primarily directed to terminal (telopeptide) antigenic sites, and not to helical or central sites¹⁸.

Membrane-bound collagen may be unique to fibroblasts in a manner analogous to membrane-bound Ig being unique to B lymphocytes. This is not to imply that other cells cannot make collagen¹⁹. The membrane-bound collagen is mobile and temperature sensitive; its membrane mobility is partially inhibited by vinca alkaloids, suggesting a role for microtubules²⁰ in the mobility of membrane-bound collagen. The presence of membrane-bound collagen seems to be a useful criterion for defining fibroblasts and should be at least as accurate as currently existing morphological criteria. The membrane definition possibly has the additional advantage of defining subpopulations of fibroblasts in so far as collagens from different organs, such as skin or cartilage, are structurally different²¹ and have different antigenicities²². These differences may be found in membrane-bound collagen, analogous to the phenomenon of allelic exclusion in B cells²³.

The presentation and mobility of collagen in the cell membranes of fibroblasts suggest several possible events in the biology of these cells. Capping of collagen may lead to the secretion of fibroblast cell product into the intracellular matrix. This theoretical consideration is strengthened by a recent observation that anti-fibroblast antibodies stimulate the secretion of trichloroacetic acid-precipitable cell product by fibroblasts²¹. Also, antibodies to chemically coupled substances on fibroblast cell membranes have been shown to cause a type of immunostimulation as measured by the incorporation of tritiated thymidine²⁴. The presence of collagen in cell membranes could place fibroblasts at risk for immunologically mediated cytotoxic or suppressor reactions. We are studying these possibilities.

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Endotoxin-sensitive membrane component of human platelets

GRAM-NEGATIVE bacteria produce endotoxin and cause intravascular coagulation, shock and ultimate death of an estimated 25% of patients with bloodstream infections^{1,2}. The number of blood platelets in such patients decreases³. In animal experiments, endotoxin aggregates platelets, induces release of vasoactive amines and adenine nucleotides, and unmasks the phospholipid procoagulant (platelet factor 3, PF3), which accelerates blood clotting⁴. To determine the mechanism of endotoxin action in man we studied the endotoxin interaction with human platelets. Here we describe experiments which demonstrate that the endotoxin-sensitive component (receptor) is present on the membrane of human platelets. Two types of changes resulting from endotoxin interaction with this receptor have been noted: selective release of 5-hydroxytryptamine (5-HT) and unmasking of platelet phospholipid (PF3).

Human platelets rendered free of plasma proteins (see Table 1) were sensitive to endotoxin. Addition of endotoxin in the form of *Salmonella enteritidis* lipopolysaccharide B (Difco, Detroit, Michigan) at a concentration of 200 µg per ml human platelets caused release of ³H-5-HT. In 21 separate experiments release of 5-HT ranged from 37% to 81% with a mean of 59 ± 2.8%. This was significantly more (*P* < 0.01) than release of 5-HT from human platelets incubated in the same conditions without endotoxin 21 ± 0.8%. 5-HT release from the control platelets can be explained by the observation that newly absorbed 5-HT behaves usually as endogenous amine, although it has a tendency to be somewhat more preferentially released⁷. In the protein-free medium used here this phenomenon could be accentuated notwithstanding the fact that gel-filtered platelets seem to preserve their functional and ultrastructural integrity⁸. In these conditions the amount of 5-HT released by endotoxin consistently exceeded two or three times control values. Release of 5-HT was selective as the cytoplasmic marker enzyme, lactate dehydrogenase, was not liberated indicating that no significant lysis of human platelets occurred. The lysosomal marker enzyme, β glucuronidase was also not released. 5-HT release was, however, paralleled by release of adenine nucleotides, determined spectrophotometrically⁹. This suggests that platelet-dense granules containing 5-HT and adenine nucleotides were

Table 1 Effect of homogenate of human platelets or their membrane-rich preparation on endotoxin-induced ^3H -5-HT release from autologous platelets

Reaction mixture	% ^3H -5-HT release (mean $\pm$ s.e.m.)
Endotoxin + buffer + platelets	66.0 $\pm$ 2.1
Endotoxin + homogenate* + platelets	46.6 $\pm$ 2.8†
Endotoxin + MRP + platelets	45.7 $\pm$ 0.7†
Buffer + homogenate + platelets	28.3 $\pm$ 1.3
Buffer + platelets (control)	24.6 $\pm$ 1.4

Human platelets, collected from fasting venous blood of healthy individuals who had not ingested aspirin or aspirin-like drugs during the preceding 8 d, were labelled with ^3H -5-HT added to platelet-rich plasma. Platelets were freed from plasma proteins and from unbound ^3H -5-HT by gel filtration according to the modified procedure of Tangen *et al.*⁶ using a column containing Sepharose 2B. Platelets containing ^3H -5-HT were eluted in the void volume of the column and plasma proteins were distinctly separate from platelets in the elution profile. Separation of platelets from plasma proteins was further documented immunochemically using a panel of antisera to whole human serum, human gamma globulin, C3 component of complement and properdin factor B (C3 activator). Gel-filtered platelets gave negative results with these antisera in the rocket immunoelectrophoresis⁸, whereas fractions containing plasma proteins were consistently positive.

*Homogenate was prepared by six cycles of freezing and thawing of gel-filtered human platelets not labelled with ^3H -5-HT.

MRP (membrane-rich pellet) was prepared from homogenate by centrifugation at 20,000g for 20 min and resuspending in 1 ml Tris-buffered saline pH 7.4. MRP was free of lysosomal β glucuronidase and lactate dehydrogenase, which were released to soluble fraction (supernatant).

Endotoxin (200 μg) was preincubated with 1 ml homogenate or MRP for 30 min at 37 °C and then gel-filtered autologous platelets labelled with ^3H -5-HT were added. After 30 min of incubation samples were centrifuged at 15,000g for 1 min at 0 °C and radioactivity in the supernatant was determined.

†Difference significant compared with reaction mixture containing endotoxin, buffer and platelets: $P < 0.01$.

selectively responding to endotoxin-induced membrane changes.

To establish that endotoxin effect on human platelets was specific, zymosan, another particulate material derived from yeast cell wall, and human erythrocyte stroma as a membrane particulate material were compared in concentrations similar to that of endotoxin. In contrast to endotoxin, neither zymosan nor red cell stroma caused a significant release of ^3H -5-HT from human platelets rendered free of plasma proteins. These data suggest that endotoxin has a specific affinity for the membrane of human platelets inducing a selective release reaction.

Experiments were carried out to determine whether the ability of human platelets to bind endotoxin plays a part in this interaction. Endotoxin was preincubated with a homogenate of unlabelled human platelets or with buffer. Then, fresh autologous human platelets labelled with ^3H -5-HT were added and ^3H -5-HT release was measured as an index of endotoxin activity. Human platelet homogenate significantly inhibited endotoxin interaction with intact platelets (Table 1) measured by release of ^3H -5-HT ($P < 0.01$). When the membrane-rich fraction sedimented from human platelet homogenate was tested, a similar inhibition of endotoxin-induced release was observed. These experiments suggest that human platelets contain an endotoxin-binding component (receptor) associated with their membrane.

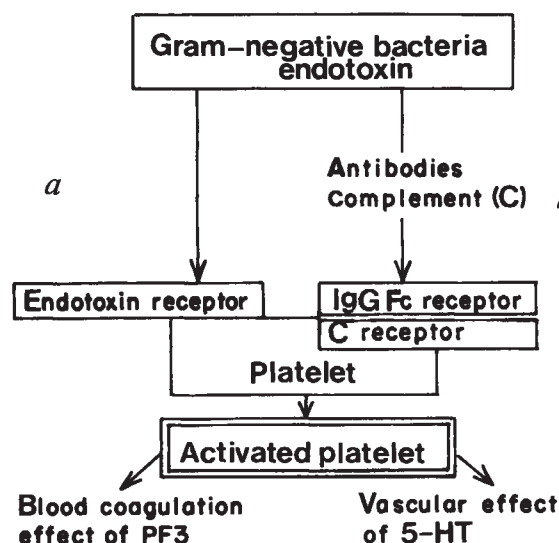
Interaction of endotoxin with human platelet receptor induced not only selective release of ^3H -5-HT but also unmasked platelet phospholipid (PF3), thus accelerating blood clotting. Evolution of PF3 activity in human platelets was measured by shortening of the Stypven time¹⁰. Human platelets were incubated for 30 min at 37 °C with endotoxin or with buffer and then the mixture was added to the autologous platelet-poor plasma in which Stypven time was measured. In three separate experiments endotoxin incubated with human platelets caused a significant ($P < 0.01$)

shortening of the Stypven clotting time of human platelet-poor plasma (34.5 ± 1.2 s) compared with plasma to which human platelets incubated without endotoxin were added (46.6 ± 2.3 s).

As PF3 is associated with platelet membrane phospholipids¹¹ its evolution represents another example of platelet membrane-related biological activity of endotoxin.

Endotoxin seemed to interact directly with a sensitive component of human platelet membrane. Human platelets rendered free of immunochemically detectable plasma proteins, were treated with trypsin (Nutritional Biochemical, 2 $\mu\text{g ml}^{-1}$), known to destroy IgG and complement adsorbed to platelets^{12,13}. This did not abolish the ability of human platelets to interact with endotoxin. Addition of heparin or hirudin as inhibitors of thrombin in concentration of 10 IU ml^{-1} did not interfere with endotoxin-induced release of ^3H -5-HT. This provided further evidence that adsorbed plasma proteins, if present, were not required in our experimental system. Second, experiments with neutralisation of the endotoxin effect by a membrane-rich preparation obtained from autologous human platelets suggest the existence of an endotoxin-binding component (receptor) on the human platelet membrane. When the receptor is triggered by endotoxin, release of vasoactive amine, 5-HT, and evolution of clot-promoting PF3 activity ensues. Thus, in addition to the well known indirect pathway of activation of human platelets by microbial antigens complexed with antibody and/or complement and interacting with platelets through receptors for IgG Fc fragment and complement^{14,15}, there is a direct pathway of human platelet activation as a result of the existence of a specific endotoxin-sensitive component (receptor) (Fig. 1).

Endotoxin-binding component (receptor) may be added to the list of previously reported receptors on the human platelet membrane. They include receptors for ADP (ref. 16), 5-HT (ref. 17), IgG fragment⁹ and thrombin-sensitive protein¹⁸. The presence of an endotoxin-binding component on cells of human origin is not limited to the platelets. Endotoxin is known to interact directly, in the absence of serum, with polymorphonuclear leukocytes¹⁹ and with red blood cells, to which it can be bound²⁰. Endotoxin-sensitive component (receptor) on human platelets as demonstrated in our study is important as platelets are intimately involved in the activation of blood coagulation, and their interaction with endotoxin may help us to understand better endotoxin-induced intravascular coagulation and shock in man.

Fig. 1 Pathways of human platelet activation by endotoxin from Gram-negative bacteria. *a*, Direct pathway; *b*, indirect pathway.

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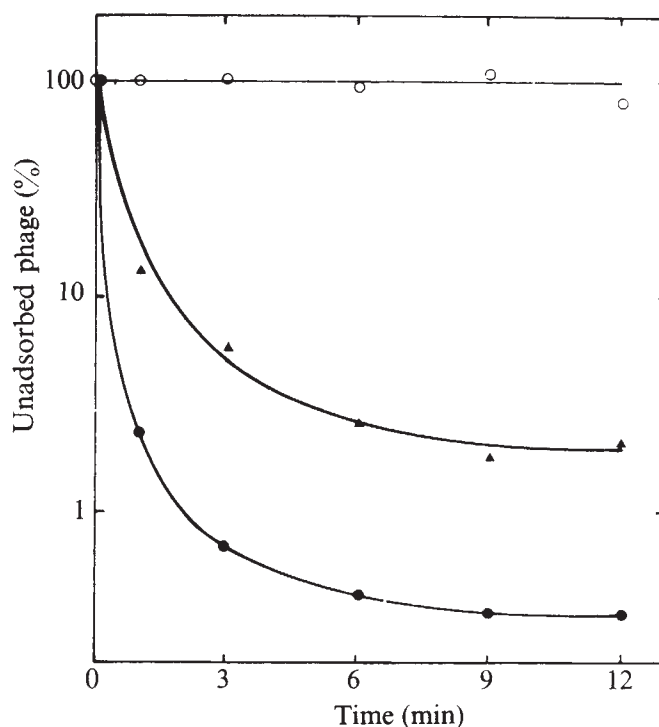


Fig. 1 Effects of D-glucose and D-fructose on bacteriophage T4 adsorption to *E. coli* B. Washed bacteria (5×10^8 cells ml^{-1}) and phage T4 (10^7 phage ml^{-1}) were mixed in 0.01 M Tris buffer, pH 7.8, containing 0.1 M NaCl at 30 °C. At intervals samples were centrifuged to remove bacteria and unadsorbed phage titred. ●, No sugar added; ○, 0.6 M D-glucose added; ▲, 0.6 M D-fructose added.

Characterisation of the bacteriophage T4 receptor site

THE fundamental importance of specific cellular interaction is indicated by its ubiquity in biological systems. Such specificity must be mediated by macromolecular recognition of intrinsically the same nature as enzyme-substrate binding and antigen-antibody interactions, but few systems involving recognition between two cells or two organisms are readily amenable to investigation at the molecular level. The attachment of bacteriophage to its host is an exception, and the chemical constitution of the specific receptor site involved in the initial binding of phage T4 to *Escherichia coli* B has therefore been examined.

The T-even bacteriophages adsorb to their host bacteria initially by the tail fibres¹, and there are strong indications that this interaction determines the specificity of adsorption and thus delineates the possible host range of the phage²⁻⁴. The initial receptor site for phage T4 is situated in the host lipopolysaccharide⁵⁻⁷, but apart from the demonstration that T4-resistant *E. coli* mutants lack UDPG pyrophosphorylase^{8,9} the receptor has not been characterised further. The *E. coli* B lipopolysaccharide consists only of the core region, and a recent analysis¹⁰ indicates that in this strain the major carbohydrate components of the core lipopolysaccharide are heptose, glucose and glucosamine; some pentose was also detected. An assay was devised to determine which of these components acts as receptor site for phage T4, and its linkage to the rest of the lipopolysaccharide.

The interaction of T4 with washed *E. coli* B cells was determined in the simplest medium which permits adsorption, in the presence of a variety of mono-, di- and tri-saccharides. Any sugar mimicking the whole or part of the phage receptor site should inhibit adsorption of the phage to the cell wall. Sugars unrelated to the receptor would have little effect. A similar approach has been used in studying the antigenic determinants of complex polysaccharides, but it has not hitherto been applied to analysis of specific interactions between organisms.

The effects of fructose, which is not a component of the *E. coli* cell wall, and glucose on adsorption of T4 are illustrated in Fig. 1. Glucose completely inhibits the adsorption, while the effect of fructose is much less dramatic and apparently nonspecific. Glucose or a related molecule therefore constitutes an essential part of the phage receptor site.

In order to determine the exact structural specifications at this site, the effects on phage adsorption of some of the epimers and derivatives of glucose were examined (Table 1). The ineffectiveness of mannose as an inhibitor indicates that the configuration at C-2 must be specifically that of glucose. Similarly the inability of galactose to inhibit binding demonstrates the importance of the configuration at C-4. Xylose had no effect on adsorption and although glucuronic acid did inhibit binding it was less effective than glucose, suggesting an absolute requirement for the C-6 group and a preference for $-\text{CH}_2\text{OH}$ rather than $-\text{COOH}$ at this position. Substitution or replacement of the glucose hydroxyl groups, unlike configurational change, frequently gave molecules which were effective antagonists of phage binding. This is true of the C-1, C-2 and C-3 positions, for glucosamine, N-acetylglucosamine, 2-deoxyglucose, 3-O-methylglucose and 1:5-gluconolactone all completely inhibited adsorption. Thus an aldohexose with configurations at C-2 and C-4 which are specifically those of glucose is the essential unit constituting the phage T4 receptor site. Glucose is the only naturally occurring sugar in this configuration, and it may be present as the hexose, glucosamine or N-acetylglucosamine, or as some more unusual derivative substituted at C-1, C-2 or C-3.

Although glucose is probably a component of all lipopolysaccharide molecules¹¹, bacteriophage T4 has an extremely narrow host range and does not adsorb to many Gram-negative organisms otherwise capable of supporting its growth¹². On the basis that this specificity probably arises from the presence of a particular linkage in the lipopolysaccharide, the effects of a variety of di- and tri-saccharides

Table 1 Effects of mono-, di- and trisaccharides on adsorption of phage T4 to *E. coli* B

Monosaccharide	Inhibition of adsorption (%)	Di- or trisaccharide	Inhibition of adsorption (%)
D-Glucose	100	Sucrose	100
D-Galactose	<1	Trehalose	100
D-Mannose	<1	Maltose	100
D-Xylose	<1	Turanose	100
D-Glucosamine	100	Kojibiose	100
N-Acetyl-D-glucosamine	100	Cellobiose	<1
2-Deoxy-D-glucose	100	Lactose	<1
3-O-Methyl-D-glucose	100	Melezitose	100
1:5-D-Gluconolactone	100	Raffinose	<1
D-Glucuronic acid	10		

Adsorption curves (Fig. 1) were determined in the presence of 0.6 M sugar, after readjustment of pH to 7.8 when necessary. In all cases a plateau was reached between 6 and 12 min, and inhibition of adsorption was calculated from the number of unadsorbed phage at 12 min.

containing glucose were investigated (Table 1). Only those sugars in which glucose is α -linked to another residue inhibited adsorption; a β -linkage at C-1, as in cellobiose, rendered the molecule inactive. Moreover, linkage of a sugar residue to the C-4 or C-6 position of the glucose component in lactose and raffinose also destroyed activity. It appears that the glucose or glucose derivative acting as receptor for T4 must be α -linked to the rest of the lipopolysaccharide and cannot be bonded to another sugar residue through either the C-4 or C-6 hydroxyl groups.

Inhibition of binding should be more effective the closer the structure of the sugar inhibitor is to that of the receptor. Sugars which inhibit binding fell into three classes in terms of the dependence of degree of inhibition on sugar concentration (Fig. 2). All monosaccharides tested—glucosamine, N-acetylglucosamine, 2-deoxyglucose, 3-O-methylglucose and 1:5-gluconolactone—displayed the same concentration effect as glucose; the dependence of inhibition on sugar concentration did not therefore indicate whether glucose or one of its derivatives was the basic component of the T4 receptor site. Maltose was slightly more effective, and sucrose, turanose, kojibiose, trehalose and melezitose all inhibited binding at significantly lower concentrations than glucose. It seems that the nature and number of the sugars to which glucose is linked do not affect its activity as an inhibitor, but that the α 1 $\rightarrow$ 4 linkage of glucose to glucose in maltose results in a less favourable configuration of the

inhibitor than the α 1 $\rightarrow$ 1 or α 1 $\rightarrow$ 2 linkage of glucose to glucose and the α 1 $\rightarrow$ 2 or α 1 $\rightarrow$ 3 linkage of glucose to fructose found in more effective inhibitors. Apparently, therefore, the glucose, glucosamine or closely related residue which acts as the T4 receptor may be in a terminal position α -linked to the 2- or 3-position of the adjoining residue, or it may be internally situated in the carbohydrate chain through linkages to its own C-2 or C-3 position. Determination of the exact structure of the receptor in *E. coli* B awaits sequencing of its lipopolysaccharide, but the value of this type of technique for partial determination and comparison of both phage receptors and bacterial surface structures is evident, as is its possible application to a wide variety of problems of intercellular recognition.

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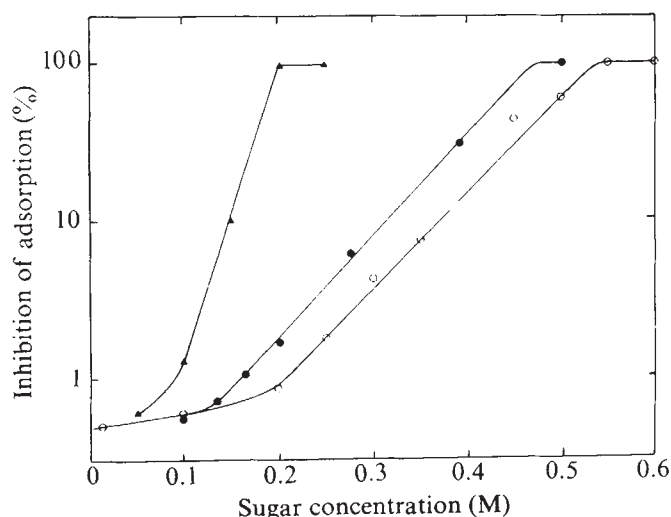
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Fig. 2 Dependence of adsorption inhibition on sugar concentration. Adsorption curves (Fig. 1) were determined at different sugar concentrations, and inhibition of adsorption calculated from the number of unadsorbed phage at 12 min (Table 1). ○, D-glucose; D-glucosamine, N-acetyl-D-glucosamine, 2-deoxy-D-glucose, 3-O-methyl-D-glucose and 1:5-D-gluconolactone also gave this concentration curve. ●, Maltose; ▲, sucrose; the same curve was obtained with turanose, kojibiose, trehalose and melezitose.



Experimental autoimmune myasthenia induced in monkeys by purified acetylcholine receptor

MYASTHENIA GRAVIS (MG) is a neuromuscular junction disease of man characterised by muscular weakness and fatigability.

Evidence that an autoimmune response to the post-synaptic nicotinic acetylcholine receptor (AChR) is important in the pathogenesis of MG has recently been presented. We have demonstrated a specific sensitisation of lymphocytes from MG patients when cultured *in vitro* with an AChR fraction extracted from the electric organ of the electric eel¹; we have further shown reduction of such sensitisation in lymphocytes from patients under steroid treatment displaying clinical improvement². Fambrough *et al.*³ have shown that there is a reduction in the number of junctional acetylcholine receptors in myasthenic muscles. Patrick and Lindstrom⁴, Heilbronn *et al.*⁵ and Aharonov *et al.*⁶ induced experimental autoimmune myasthenia in rabbits by injection of AChR isolated from electric organ

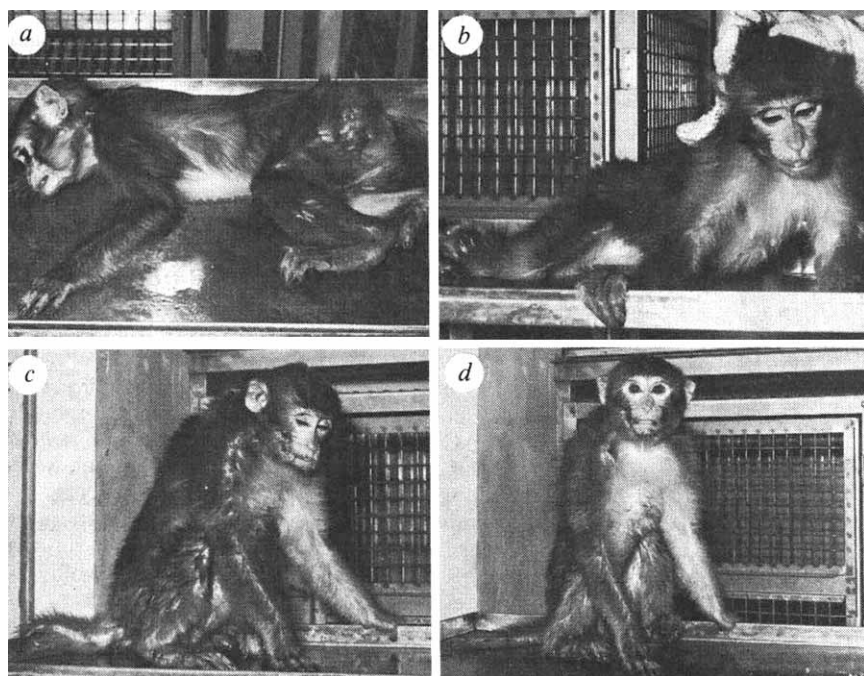


Fig. 1 Myasthenic monkey one week after fourth injection of purified acetylcholine receptor. *a, b*, Before injection of Prostigmine; *c, d*, 5 min after injection of Prostigmine (0.2 mg); *d*, 10 min after injection of Prostigmine.

tissue of either the electric eel, *Electrophorus electricus* or the electric ray, *Torpedo*.

Here we describe the induction of experimental autoimmune myasthenia gravis in Rhesus monkeys by injection of AChR purified from the electrogenic tissue of *T. californica*. The clinical, pharmacological electrophysiological, immunological and histological findings in monkeys suffering from experimental autoimmune myasthenia closely parallel those in patients suffering from MG.

Purified AChR from electrogenic tissue of *T. californica* was obtained by solubilisation of membrane fragments with 1% Triton X-100, followed by affinity chromatography on a *Naja naja siamensis* neurotoxin-Sepharose resin, as described by Aharonov *et al.*⁶, with slight modifications, for isolation of AChR from the electric eel. The specific activity of the purified AChR was 8,000–10,000 pmol toxin bound per mg protein.

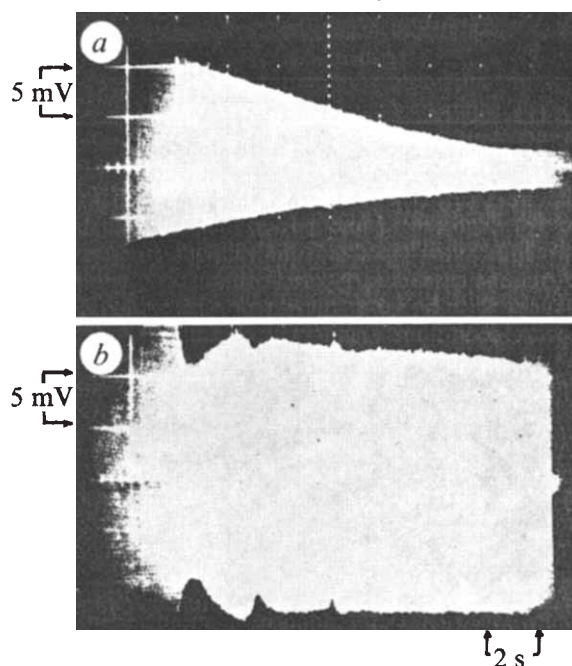
Two female Rhesus monkeys, *Macaca mulatta* (3 kg), were injected in both foot pads and intradermally in 3–4 spots with 100–200 μ g AChR in 0.5 ml saline mixed with 0.7 ml Freund's complete adjuvant (FCA). At three-week intervals, both animals were subsequently challenged three times with AChR administered in the same way. Control monkeys received FCA alone. Within two weeks of the third injection of AChR, the two monkeys showed signs of fatigue, hypoactivity, anorexia and weight loss. One week after the fourth injection the monkeys' condition was acute, with extreme flaccid paralysis of the limbs and trunk, sinking of the head and severe difficulties in breathing (Fig. 1*a* and *b*). Bulbar signs included ptosis, external ophthalmoplegia, facial diplegia and paralysis of the jaw muscles, with uncontrolled salivation and severe dysphagia. Shortly after intramuscular injection of 0.2 mg of the anticholinesterase Prostigmine (neostigmine bromide) the sick monkeys began to display signs of recovery which was complete within ten minutes: they became erect, raised their heads, placed their legs under their bodies, were able to move and essentially to breathe normally; at the same time, all bulbar signs disappeared (Fig. 1*c* and *d*). This improved condition was maintained for about 4 h. No clinical changes were observed in monkeys injected with FCA alone.

Neurophysiological studies were made on the animals after 6 weeks, before the fourth injection, using a Grass electromyograph with stimulator unit and differential ampli-

fier. A coaxial recording electrode was introduced into the calf muscle and a bipolar stimulation electrode was placed on the buttock over the sciatic nerve. The electromyographic examination revealed a decrease in amplitude during repetitive nerve stimulation in AChR-injected monkeys (Fig. 2*a*) and a normal response in control animals (Fig. 2*b*).

After the pharmacological investigations and before the animals were killed, blood was collected by heart puncture and humoral antibodies against purified AChR were detected by the agar gel immunodiffusion assay in the sera of the animals (see Fig. 3). Cellular sensitivity towards

Fig. 2 Electromyographs from myasthenic and normal monkeys. Tetanic stimulation was carried out at a frequency of 40 s⁻¹, with pulses of 0.1 ms duration and 150 V amplitude. Horizontal bar, 2 s; vertical bar, 5 mV. *a*, Myasthenic monkey; *b*, Control monkey.



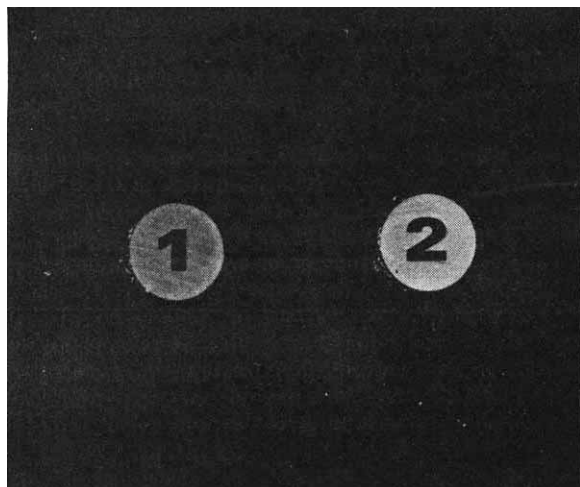


Fig. 3 Agar gel immunodiffusion of serum from a sick monkey. Well 1, monkey serum; well 2, purified acetylcholine receptor ($300 \mu\text{g ml}^{-1}$).

AChR was demonstrated in lymph node cells using the lymphocyte transformation technique ($2\text{-}^{14}\text{C}$ -thymidine uptake) according to the method described by Tarrab *et al.*⁷. The results are expressed as stimulation indices (SI) (the ratio of radioactivity in tubes containing antigen to the radioactivity in control antigen-free tubes), and they represented an average of values obtained in duplicate cultures. Lymphocytes of myasthenic monkeys showed SI of 3.0, 12.5 and 12.8 when incubated in the presence of $0.5 \mu\text{g}$, $1 \mu\text{g}$ and $2 \mu\text{g}$ AChR, respectively, and SI of 0.8–1.4 when incubated with other antigens such as rabbit actin, rat collagen and lysozyme. No stimulation was obtained in the control animals with either AChR (SI 0.9–1.3) or the other antigens (SI 0.8–1.4).

Samples of brain, thymus and muscles were taken for pathological examination. The formalin-fixed paraffin-embedded sections were stained with haematoxylin–eosin and light green. The histopathology revealed a few lymphocytes between muscle cells in the leg muscle and mild lymphorrhage foci in a buccal muscle (Fig. 4).

The clinical picture and the histopathological findings, as well as the characteristic clinical improvement after anticholinesterase administration and the pathognomonic decremental response to repetitive nerve stimulation, indicate that the monkeys suffered from myasthenia. The criteria for diagnosis of MG in patients suffering from criteria applied are closely analogous to the principal muscular weakness.

Fifteen years ago it was already suggested that MG

Fig. 4 Lymphorrhage focus in a buccal muscle section from a myasthenic monkey. Haematoxylin–eosin and light green. $\times 280$.



might be an autoimmune disease of the neuromuscular junction^{8,9}. Goldstein and coworkers¹⁰, Kalden *et al.*¹¹ and Kawanami and Mori¹² attempted to provide an experimental model for the disease in experimental animals injected with either muscle or thymic extracts. Although electrophysiological evidence for a partial neuromuscular block was demonstrated, the animals did not show any clinical signs of muscle paralysis, and others were unable to confirm these findings^{13–15}. Clinical as well as electrophysiological and pharmacological signs of myasthenia were recently induced by Patrick and Lindstrom⁴ and subsequently by Heilbronn *et al.*⁵ and ourselves⁶ in rabbits immunised with AChR purified from electric fish. The nicotinic AChR of the electrogenic organ tissue of the electric fish is pharmacologically similar to the same receptor in skeletal muscle¹⁶, and cross reacts immunologically with AChR from other species^{4,17} and, as the present study suggests, most probably with monkeys as well. These findings thus provide supporting evidence that MG is an autoimmune disease and that AChR is the auto-antigen. Our earlier observations^{1,2} on a cellular immune response to AChR of lymphocytes from patients with MG provided direct evidence that breakdown of tolerance to self-AChR is involved in the neuromuscular block. The AChR-induced myasthenia in monkeys, so similar to the human disease, provides a valuable experimental model for studying both the mechanism and therapy of myasthenia gravis.

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Anti-fertility drugs: novel non-hormonal compounds that inhibit prostaglandin metabolism

PREGNANCY has been terminated in animals and/or humans by oestrogens^{1–3}, androgens^{4,5}, anti-oestrogens^{6,7}, prostaglandins^{8–10}, and various non-steroidal non-hormonal agents^{6–11}.

Table 1 Subcutaneous doses of L10492 and L10503 for each of 5 consecutive days required for termination of pregnancy in 100% rats and hamsters

Animal	Treatment on gestation days	L10492 (mg kg ⁻¹ d ⁻¹)	L10503 (mg kg ⁻¹ d ⁻¹)
Rat	1-5	25.0	100.0
	4-8	10.0	30.0
	6-10	2.5	20.0
	8-12	10.0	30.0
Hamster	1-5	2.0	0.5
	4-8	0.5	0.25
	6-10	3.0	1.5
	8-12	> 10.0	> 5.0

The mechanism of action of these agents has not been defined and effectiveness varies with the species. Elevated levels of endogenous prostaglandins (PGs) may be associated with toxæmia of pregnancy and habitual abortion¹². PG may also alter foetal and placental blood flow^{13,14}. A compound that inhibits PG metabolism—thereby elevating PG levels—could possibly interrupt pregnancy. This report describes a new class of non-hormonal compounds, which are 100% effective as anti-fertility agents in several species of animals, and also outlines studies on the effect of these compounds on PG synthesis and metabolism in rat placenta.

Compounds studied were 2-(3-methoxyphenyl)-5-H-s-triazolo [5,1- α] isoindole (L10492) and 2-(3-methoxyphenyl)-5,6-dihydro-s-triazolo [5,1- α] isoquinoline (L10503). These compounds in sesame oil were administered subcutaneously to female Sprague-Dawley rats and Golden Syrian hamsters on various days after mating, the rats and hamsters were killed on days 16 and 14 of pregnancy, respectively, and the uteri examined for live or dead foetuses, implantation sites or resorpting foetuses.

Dose-related anti-fertility effects were obtained with both compounds when administered in various 5 d segments during the first half of gestation in both species (Table 1). The most effective time for treatment was during gestation days 6-10 in rats and days 4-8 in hamsters. Although 100% pregnancy termination was obtained in each treatment period, the most effective time for treatment was soon after implantation of the fertilised ova. Note that L10492 was more potent in rats and that L10503 was more potent in hamsters. The latter, however, was more sensitive to treatment with either compound. Single injections of these compounds also produced dose and time of pregnancy-related abortifacient activity in both species (Fig. 1). Dose required for 100% effectiveness, however, was higher than that needed with multiple injections. The optimum time for drug administration was during days 4 or 5 in the hamster and days 7 or 8 in the rat, corresponding to the time immediately following implantation of the blastocyst in the uterus. As in the case of the multiple injection studies, the hamster was the more sensitive species and L10492 was more potent in the rat than L10503; the latter was, however, more potent in the hamster.

Studies on the mechanism of action indicated that the primary site for drug action is at the utero-placental complex. Higher brain centres, hypothalamus, pituitary and ovaries are not required for the anti-fertility activity as rats ovariectomised on day 8 of pregnancy and maintained in the gravid state by daily subcutaneous injections of 10 mg progesterone plus 1 μ g oestradiol benzoate, resorbed all their foetuses after a single 50 mg kg⁻¹ injection of L10492. Similar results were obtained in both animal species with both compounds in several different treatment regimens. In addition pregnant rats administered single (days 7 or 8 of pregnancy) or multiple (days 6-10 of pregnancy) doses of L10492 (50 mg kg⁻¹ single or 2.5 mg kg⁻¹ d⁻¹) or L10503 (100 mg kg⁻¹ single or 25 mg kg⁻¹ d⁻¹) and progesterone at daily doses of 4 mg per rat on days 6-15 of gestation, resorbed the products of pregnancy. These treatments did not affect the corpora lutea which were well developed

and apparently functional during foetal resorption. Mechanism of action does not involve ovulation inhibition as treatment was initiated after ovulation and conception (Table 1 and Fig. 1). Also rats and hamsters treated with abortifacive doses of each compound in various phases of their oestrous cycles ovulated normally. These compounds did not inhibit implantation of the fertilised ova as administration of L10492 or L10503 after ovulation and mating but before implantation allowed the ova to implant but subsequently the implants were resorbed (Table 1 and Fig. 1). Hormonal or anti-hormonal activity is not involved in the anti-fertility action of these agents as they are devoid of oestrogenic, anti-oestrogenic, androgenic, anti-androgenic, progestational or anti-progesterone activities.

The possibility that placental PG synthesis and metabolism was altered by drug treatment was investigated. Metabolism was studied as described previously¹⁵. Synthesis was studied using the method of Flower *et al.*¹⁶ and modified as described in Table 2.

Placental PG metabolism varies with the day of pregnancy and is lowest on day 8, is sharply increased on day 11, returns to a low level on day 14 and then becomes maximal on days 16 to parturition. As the optimum time for anti-fertility activity of L10503 (100 mg kg⁻¹, subcutaneously) was found to be on days 7, 8 or 9 of gestation¹⁷, we studied the effect on placental PG metabolism on days 8, 9 and 10 and on PG synthesis on day 9. L10503 inhibited PG metabolism on a per mg protein basis, the degree of inhibition being dependent on the day of drug treatment. In the case of PGE₁ there was no alteration in metabolism when the compound was administered on days 8, 9 or 10 of gestation but when treatment was on both days 9 and 10 there was a 48% inhibition of metabolism. PGF_{2 α} metabolism, however, markedly decreased when L10503 was injected on days 8, 9 or 9 and 10 but a single injection on day 10 produced no significant change in placental PGF_{2 α} metabolism. Administration of this drug on days 9 and 10 resulted in a 68% decrease in PGF_{2 α} metabolism; thus L10503 is a more potent inhibitor of PGF_{2 α} metabolism than PGE₁ metabolism. Not only did this compound affect PG metabolism but it also reduced placental weight when administered on days 8, 9 and 10 of pregnancy, the greatest effect resulting from

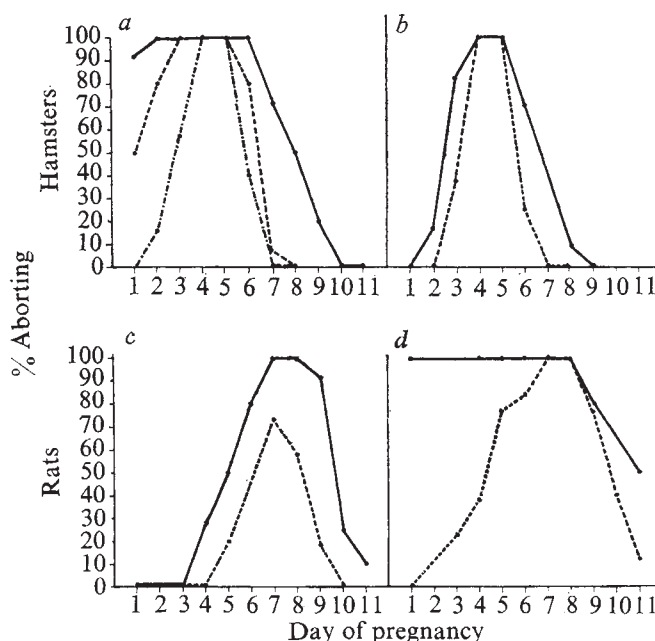
Fig 1 Abortifacient activity of single subcutaneous injections of L10503 (a, c) or L10492 (b, d) on various days during the first 11 d of pregnancy in rats and hamsters. a: —, 25 mg kg⁻¹; ---, 6.25 mg kg⁻¹; - · - · -, 1.56 mg kg⁻¹. b: —, 25 mg kg⁻¹; ---, 12.5 mg kg⁻¹. c and d: —, 100 mg kg⁻¹; ---, 50 mg kg⁻¹.

Table 2 Effect of L10503 treatment on various days of pregnancy on rat placental weight, prostaglandin metabolism and synthesis

Pregnancy day of L10503 treatment	Placental weight (mg)	PGE ₁ pmol metabolised per h		PGF _{2α} pmol metabolised per h	
		per mg protein	per organ	per mg protein	per organ
8	88.5±8.1†	140.2±9.4	855.5±57.3*	7.5±1.2*	47.3±7.4*
9	88.7±5.1†	144.2±9.6	894.3±59.7*	8.2±1.3*	52.4±8.1*
10	94.2±5.0	125.7±10.3	817.4±66.8*	14.6±3.1	99.5±21.3†
9+10	73.2±1.4*	78.5±4.1*†	392.5±20.6*§	5.9±0.7*	31.2±3.9*§
Sesame oil (9+10)	112.7±4.7	152.0±8.1	1185.6±63.0	18.2±0.6	149.6±5.3
		PGE ₂ pmol synthesised per h		PGF _{2α} pmol synthesised per h	
		per mg protein	per organ	per mg protein	per organ
9	85.7±3.7	7.50±1.51	60.8±12.5	1.90±0.17	15.4±1.4
Sesame oil (9)	113.6±5.1	8.73±1.19	95.0±12.9	1.73±0.12	18.8±1.3

Values are mean $\pm$ s.e. of three pools of placentae from 4–8 rats each. Placentae preserved at -30°C , homogenised in Bucher medium, 1:5 (w/v) for PGF_{2α} metabolism and 1:10 (w/v) for PGE₁ metabolism studies, and centrifuged at 10,000g for 15 min. To 2 ml aliquots of the supernatant were added 4 mM NAD⁺, 0.1 μM PGF_{2α} (2.25 Ci mmol⁻¹) or 0.1 μM PGE₁ (2.45 Ci mmol⁻¹). Incubation time at 37 $^{\circ}\text{C}$ was 20 min for PGF_{2α} and 5 min for PGE₁ metabolism. Enzymatic reaction was stopped by addition of 0.2 ml 1 N HCl to acidify at pH 3.0. Extraction and separation by thin-layer chromatography of prostaglandins and their metabolites (15-keto and 15-keto-dihydro derivatives) were performed as described previously¹⁵. For PG synthesis studies, tissues were homogenised with 0.1 M Tris-HCl buffer pH 8.2, 1:5 (w/v). To 2 ml aliquots were added 4 mM adrenaline, 4 mM glutathione, and 1 μM ³H-arachidonic acid (0.53 Ci mmol⁻¹). Time of incubation was 1 h and reaction was stopped by addition of citric acid to acidify at pH 3.0. Extraction and separation by thin-layer chromatography was as described previously¹⁶.

Significance between treated and control groups was determined using Dunnett's *t* test; an *F* test following analysis of variance was used to compare data between experimental groups. **P* < 0.01 and †*P* < 0.05 against vehicle control group; §*P* < 0.01 against day 9 treatment group.

treatment on both days 9 and 10. Note that even though placental weight was reduced by treatment, the concentration of protein per 100 mg tissue was unaffected (6.94 ± 0.13 mg, mean $\pm$ s.e.). PG metabolism was therefore computed on the basis of total placental weight (Table 2). Treatment on any of the three pregnancy days studied resulted in significant reductions in PGE₁ metabolism. L10503 administered on days 9 and 10 decreased PGE₁ metabolism by 67%, and an even greater suppression of PGF_{2α} metabolism was observed after single day treatments on each of the three days of pregnancy. Administration of the compound on days 9 and 10 resulted in a 79% reduction in PGF_{2α} metabolism. *In vitro* studies also showed that L10503 inhibited PGF_{2α} metabolism although PGE₁ metabolism was unaffected.

PGE₂ and PGF_{2α} synthesis in the placenta was not significantly altered when L10503 (100 mg kg⁻¹ subcutaneously) was administered on day 9 of pregnancy. PGE₂ synthesis in this tissue was four to five times greater than PGF_{2α} synthesis.

These studies on PG metabolism indicate that the mechanism of anti-fertility action of L10503 may involve the inhibition of placental PG metabolism, leading to an elevated level of endogenous PG which could be detrimental to placental physiology, that is, altered haemodynamics.

The toxicity (LD₅₀) of L10492 and L10503 is very low compared with the abortifacient doses (ED₁₀₀) when the compounds were administered for 5 consecutive days. The toxic dose was greater than 64 times and > 320 times more than the ED₁₀₀ doses for rat and hamster, respectively, in the case of L10492, and > 8 times and > 640 times more than the ED₁₀₀ doses for rat and hamster, respectively, in the case of L10503.

High but not abortifacient doses of these anti-fertility agents have not produced teratogenic effects. Animals whose pregnancies were terminated with these drugs promptly returned to normal oestrous cycles, mated, became pregnant, had normal litters and lactated normally. The offspring resulting from these pregnancies were fertile at maturity.

These studies indicate that L10492 and L10503 are safe and effective anti-fertility agents that may produce their effect by inhibiting endogenous PG metabolism in the placenta. Furthermore these compounds may be useful in elucidating the role of PGs and their metabolism in placental function.

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Non-antiviral activities of interferon are not controlled by chromosome 21

USING mouse-human cell hybrids, Tan *et al.*¹ assigned the gene(s) for the expression of the antiviral state induced by human (leukocyte) interferon to chromosome 21; furthermore, human skin fibroblasts trisomic for chromosome 21 proved more sensitive to the antiviral activity of human (leukocyte) interferon than normal diploid fibroblasts or trisomic 18 or 13 fibroblasts^{2,3}. Since the non-antiviral and antiviral activities of interferon remain inseparably linked through approximately a million-fold purification (ref. 4 and references cited therein), the question may be raised

whether these various activities of interferon are accounted for by the same mechanisms and whether the expression of the non-antiviral activity of (human) interferon is also controlled by chromosome 21.

We report here that—in comparison with normal diploid (D) cells, or trisomic 18 (T-18) or trisomic 13 (T-13) cells—trisomic 21 (T-21) cells are indeed more sensitive to the antiviral action but not to the non-antiviral effects of interferon. As parameters of the non-antiviral activity of interferon we chose 'priming' (increased responsiveness of interferon-treated cells to interferon induction by poly(I)-poly(C)^{5,6}) and 'toxicity-enhancement' (increased susceptibility of interferon-treated cells to the cytotoxicity of double-stranded RNAs^{7,8}, such as poly(I)-poly(C)). Both the antiviral and the non-antiviral activities of interferon were explored with human leukocyte and fibroblast inter-

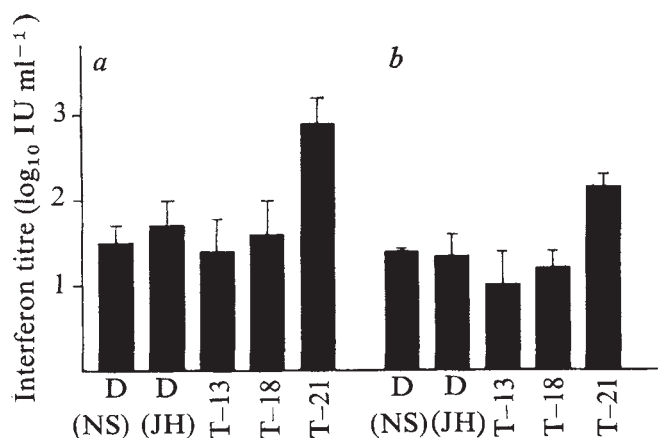


Fig. 1 Antiviral activity of human leukocyte (a) and fibroblast (b) interferon in normal diploid fibroblasts (two cell lines, designated NS and JH, respectively) and fibroblast trisomic for chromosome 13, 18 or 21. Wells of a semi-micro Linbro plate were seeded with 10^5 cells ml⁻¹ (Eagle's MEM + 10% foetal calf serum) per well. When confluent (usually after 4 d), the cells were maintained for another 3 d in EMEM + 3% foetal calf serum. The cultures were then treated with our standard leukocyte or fibroblast interferon preparations, diluted (in EMEM + 3% foetal calf serum) to give approximately $1.5 \log_{10}$ units ml⁻¹ in NS cells, for 16 h at 37 °C, drained, washed, challenged with Mengo virus and further processed by the neutral red uptake technique as described by Finter²⁷. The amounts of stain incorporated were determined by optical density readings at 542 nm. The readings made for the virus controls did not differ significantly from one cell type to another; nor did the readings made for the different cell controls. Hence, gross differences in normal cell metabolism or virus replication pattern between the different cell types employed may be excluded. Bars show means (+ s.d.) for three experiments.

feron preparations. Our studies indicate that, unlike the antiviral activity, the non-antiviral effects of human leukocyte and fibroblast interferon are not genetically determined, or at least not by chromosome 21.

Human leukocyte and fibroblast interferon were prepared according to well-established procedures^{9,10}. The leukocyte and fibroblast interferon preparations employed had specific activities of approximately 10^4 units per mg protein. Interferon titres are expressed in terms of the British Research Standard B for human interferon (69/19). Fibroblast cultures were established from skin biopsies. The cells were subcultured in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum. All cell cultures were matched as nearly as possible with regard to the number of cell generations in culture.

In agreement with Tan's findings^{2,3}, our experiments showed that T-21 cells were about ten times more sensitive

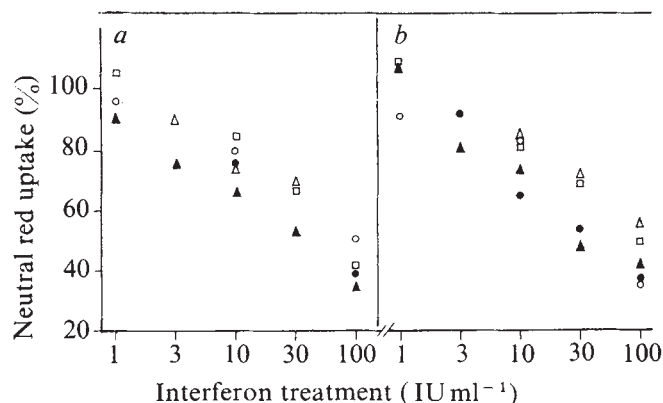


Fig. 2 Toxicity-enhancing activity of human leukocyte (a) and fibroblast (b) interferon in normal diploid fibroblasts (NS, JH) and fibroblasts trisomic for chromosome 13, 18 or 21. Semi-confluent cell cultures in 60 mm Falcon Petri dishes were treated with interferon at the indicated concentrations (in Eagle's MEM + 3% foetal calf serum: 2 ml per Petri dish) for 16 h at 37 °C, drained, washed, and then exposed to $100 \mu\text{g ml}^{-1}$ of poly(I)-poly(C) (P-L Biochemicals, Milwaukee, Wisconsin) (in Eagle's MEM, 1 ml per Petri dish) for 1 h at 37 °C. The plates were then washed and further incubated with serum-free Eagle's MEM (4 ml per Petri dish) for 16 h, at which time toxicity was monitored by neutral red uptake⁷. Symbols represent mean values for three experiments. ●, NS; ▲, JH; ○, T-13; △, T-18; □, T-21.

to the antiviral action of human leukocyte interferon than either T-13 or T-18 or normal diploid cells (Fig. 1). In addition, T-21 cells were more sensitive than T-13, T-18 or diploid cells to the antiviral action of human fibroblast interferon (Fig. 1).

The inhibitory effect of poly(I)-poly(C) on virus multiplication is thought to be mediated by interferon induction even at polymer concentrations which do not lead to the release of detectable amounts of interferon in the cell culture medium¹¹⁻¹³. If this assumption is correct, one may expect poly(I)-poly(C) to be more active in inhibiting virus replication in T-21 than in normal diploid cells. As shown before², poly(I)-poly(C) was indeed more active in T-21 than in normal diploid cells: the minimal concentration of poly(I)-poly(C) required to suppress cytopathogenicity induced by vesicular stomatitis virus was $0.003 \mu\text{g ml}^{-1}$ in T-21 cells but $0.03 \mu\text{g ml}^{-1}$ in three normal diploid cell lines (designated NS, JH and FS-4).

In accord with previous findings⁷, human fibroblast interferon enhanced the susceptibility of human fibroblasts to the toxicity of poly(I)-poly(C) (Fig. 2). The extent of cell destruction, as monitored by neutral red uptake, closely paralleled the amounts of interferon applied. Leukocyte interferon proved equally effective as fibroblast interferon in enhancing the toxicity of poly(I)-poly(C). Most importantly, all cell lines tested, whether disomic or trisomic, behaved quite similarly in their sensitivity to the toxicity-enhancing activity of interferon (Fig. 2).

Similar results were obtained with regard to the priming activity of interferon (Fig. 3): no marked differences were noted in the sensitivities of T-13, T-18, T-21 and normal diploid cells to the priming effect of either human leukocyte or fibroblast interferon.

Two major conclusions follow from these results. First, though the antiviral activity of human leukocyte and fibroblast interferon seems to be controlled by gene(s) located on chromosome 21, their non-antiviral activities are not. It is even arguable whether the non-antiviral activities of interferon are genetically regulated at all. On several occasions it has been suggested, albeit without direct experimental evidence, that the priming as well as the toxicity-enhancing effect of interferon are solely cell surface (or

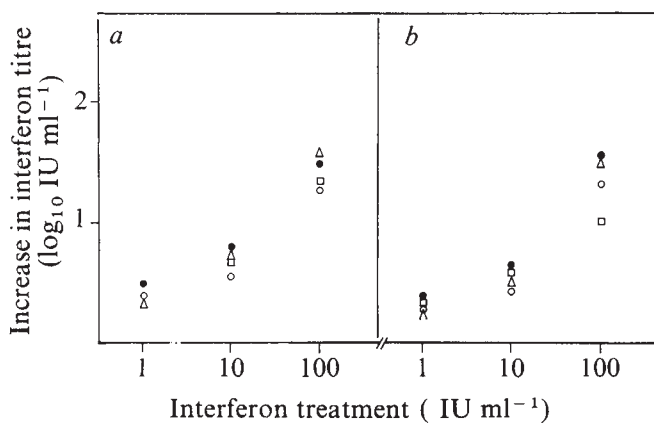


Fig. 3 Priming activity of human leukocyte (a) and fibroblast (b) interferon in normal diploid fibroblasts (NS) and fibroblasts trisomic for chromosome 13, 18 or 21. The procedure followed for measuring the priming activity of interferon diverged from that applied for measuring the toxicity-enhancing activity (legend to Fig. 2) in that confluent (instead of semiconfluent) cell cultures were used (about 5×10^5 cells per Petri dish), poly(I)-poly(C) was added at 50 (instead of 100) $\mu\text{g ml}^{-1}$, and the plates were incubated with Eagle's MEM+3% foetal calf serum (instead of serum-free medium) after their exposure to poly(I)-poly(C). The supernatant fluids collected 16 h after poly(I)-poly(C) had been removed were assayed for interferon by the dye uptake method²⁷ in human skin fibroblasts (NS) challenged with Mengo virus. The interferon titres are expressed as the differences in titre obtained between the 'primed' and control cell cultures. ●, NS; ○, T-13; △, T-18; □, T-21.

cell membrane) phenomena^{6,14,15}. The data presented here and previous data^{3,8}, eliminating the necessity of protein synthesis for the expression of the priming and toxicity-enhancing effects of interferon, would seem consistent with this hypothesis. Although the data reported here indicate fundamental differences in the mechanisms underlying the antiviral and non-antiviral activities of interferon, they do not rule out the possibility that these mechanisms only diverge from a certain point onward: for example both the antiviral and non-antiviral effects of interferon may be triggered from the cell surface¹⁶⁻¹⁸. Whereas the 'priming' and toxicity enhancement could be envisaged as the direct consequence of an alteration of the cell membrane, the expression of the antiviral state would require a few additional steps involving derepression, transcription and, finally, translation of the putative antiviral protein.

Second, human leukocyte interferon and human fibroblast interferon differ considerably in their biological properties (antiviral activity in heterologous cells¹⁹⁻²¹), physicochemical properties (stability towards heat²², shaking²³, guanidine treatment²³, reactivation by anionic detergents²⁴) and antigenic properties (as assessed by neutralisation with specific antisera^{25,26}). These differences may imply that human leukocyte interferon and human fibroblast interferon differ structurally²⁵ and/or contain distinct molecular species²⁴, and, if our interferon preparations contain different molecular species, the question arises whether the antiviral and non-antiviral activities of the interferon preparation reside in one and the same or in separate molecular species. Yet, in spite of these differences, leukocyte and fibroblast interferon exhibited similar antiviral, priming and toxicity-enhancing activities in the fibroblast cell lines studied. Hence, human leukocyte interferon and human fibroblast interferon should share a common structural determinant that initiates both the antiviral and non-antiviral responses (possibly from a common receptor site at the cell surface, as suggested above).

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Saturable, energy-dependent, transmembrane transport of prostaglandins against concentration gradients

SOME vertebrate tissues, such as the choroid plexus, anterior uvea, and the kidney cortex, accumulate radioactivity against a concentration gradient when incubated with ^3H -prostaglandins (^3H -PGs)¹. This accumulation is reversible, temperature-dependent and can be inhibited by metabolic and transport inhibitors, and by high concentrations of PGs, PG analogues and some organic acids such as probenecid and indomethacin^{2,3}.

It has been argued¹ that *in vitro* accumulation of PGs by the choroid plexus and anterior uvea reflects the transmembrane transport of these biologically active fatty acids across the blood-brain and blood-ocular fluid barriers. Such active PG absorption from the extracellular fluids of brain and eye could have considerable physiological significance as these potentially harmful autocoids are produced, but not effectively metabolised, by intraocular⁴ and central nervous tissues⁵. Recent experiments have shown that rabbit erythrocytes are impermeable to PGs of the E and F series, indicating that cellular membranes can represent effective barriers to the free diffusion of these autocoids⁶. We must consider therefore that the elimination of PGs from the extracellular fluids of eye and brain, the intracellular metabolism of PGs by the lung, and their excretion by the kidneys require specific transmembrane transport processes⁷.

In vivo experiments have already provided evidence that

PGs are transported out of the intraocular⁸ and cerebrospinal⁹ fluids and are absorbed from the vaginal lumen¹⁰ of rabbits by facilitated, carrier-mediated mechanisms. None of these *in vivo* experiments, however, could provide direct evidence for active transport of chemically unaltered PGs as the rapid metabolism of PGs by lung and liver prevents the chemical identification of the transported PGs and the maintenance of constant PG concentration in the blood. Whereas *in vitro* studies demonstrated that PG uptake against a concentration gradient is not primarily associated with a chemical alteration of the PG

molecule¹¹, such uptake studies could not distinguish between PG adsorption on to specific binding sites and active transport of free PGs into interstitial fluid compartments.

In the present experiments, a bladder-like preparation of the isolated rabbit vagina was used as a model system to demonstrate that chemically unaltered PG can indeed be transported across a biological membrane against a chemical concentration gradient by a temperature and energy-dependent, saturable process.

Either the inside (M_i) or the outside (M_o) or both media contained ^3H -PGF_{2 α} or ^3H -PGE₁ (New England Nuclear) and both sides were aerated with 95% O₂, 5% CO₂.

In experiments in which initially only M_i contained ^{14}C -sucrose and ^3H -PGF_{2 α} , there was a rapid entry of ^3H into M_o , but there was no significant loss of ^{14}C from M_i or detectable entry of ^{14}C into M_o . The results of a typical experiment are shown in Fig. 1a. In four identical experiments, the observed $M_i \rightarrow M_o$ ^3H transfer corresponded to a total apparent PGF_{2 α} flux of 400 ng h⁻¹. Thus, the rabbit vagina, presumably its epithelial lining, allows a rapid flux of PGF_{2 α} in the outward direction, yet it is a permeability barrier to sucrose, a substance of essentially identical molecular weight.

When initially only M_o contained ^3H -PGF_{2 α} , there was an initial decrease in ^3H activity of M_o in all five such experiments, presumably due to the entry of ^3H -PGF_{2 α} into the tissue itself, but there was no detectable entry of ^3H activity into M_i . The result of a typical experiment of this series is shown in Fig. 1b. Thus, the rabbit vagina is an effective barrier to the influx of PGF_{2 α} into the lumen while allowing a large PGF_{2 α} efflux from the lumen to the outside. This outward ^3H flux could not be caused by passive ^3H -PGF_{2 α} diffusion only, as after the first 90 min of incubation, the ^3H concentration in M_o surpassed that of M_i (Fig. 1a).

When the vagina was inverted before insertion of the cannula, the direction of the net ^3H flux remained 'mucosal' $\rightarrow$ 'serosal'. Experiments with ^{14}C -linoleic or ^{14}C -octanoic acid showed no evidence of transmembrane transport of these unrelated fatty acids.

When initially both M_o and M_i contained the same ^3H -PGF_{2 α} concentration, the ^3H activity of M_i steadily decreased, whereas that of M_o showed a continuous increase between 10 and 60 min of incubation. This resulted in the establishment of a continually increasing ^3H gradient across the isolated vagina reaching a more than 18-fold gradient by 2 h incubation at 37 °C (Fig. 2a). The mean ^3H gradient in five such experiments was 5.2 ± 0.8 ($n = 13$) at the end of 1 h, reaching a peak of 14 ± 4 ($n = 4$) at 3 h incubation at 37 °C. Similar results were obtained when ^3H -PGE₁ was used instead of ^3H -PGF_{2 α} , but the extent of the maximum ^3H gradient was lower (three to sevenfold between 2 and 4 h incubation).

At 4 °C, the ^3H activity of M_o remained equal to M_i until the temperature was raised to 37 °C (Fig. 2a). When both media contained 2 mM iodoacetate, and N₂ in the aeration mixture, net flux of ^3H was not observed, and M_o/M_i remained at unity indefinitely at 37 °C (Fig. 2a). The $M_i \rightarrow M_o$ flux was partially inhibited by 10^{-5} M, and was almost completely blocked by 10^{-4} M PGF_{2 β} (Fig. 2b). PGF_{2 β} , a relatively inactive analogue of PGF_{2 α} (ref. 12) was found to be more effective than PGF_{2 α} itself, yielding essentially complete inhibition of ^3H -PGF_{2 α} transport at 10^{-6} M. The unrelated fatty acid, octanoic acid, produced partial inhibition only at a concentration of 10^{-3} M.

To determine how much of the ^3H activity transported across the vagina remained associated with the original PG, both M_o and M_i were removed after 90 or 120 min of incubation and extracted for PGs (ref. 13). Paper chromatography¹⁴ of such extracts showed that only about two-thirds of the ^3H activity of M_o remained associated with PGF_{2 α} , whereas approximately one-fourth of the ^3H was found with the 15-keto-PGF_{2 α} spot. For this reason 10^{-4} M furosemide was used in all further experiments to inhibit the 15-dehydrogenase activity¹⁵. In the presence of furosemide the ^3H gradient

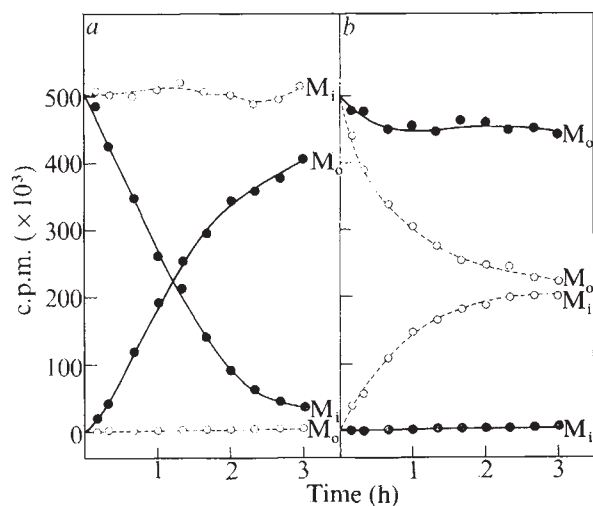


Fig. 1 Unidirectional ^3H and ^{14}C fluxes across isolated rabbit vagina. *a*, M_i to M_o fluxes. Initially M_i contained ^3H -PGF_{2 α} (●) plus PGF_{2 α} (total [PGF_{2 α}] = 10^{-6} M) plus ^{14}C -sucrose (○), whereas M_o contained no PG or radioactivity. Rapid outward movement of ^3H activity reversed initial ^3H gradient within 90 min. *b*, M_o to M_i fluxes. Initially M_i contained no radioactivity and no PG whereas M_o contained ^3H -PGF_{2 α} (as above; ●) plus ^{14}C -thiourea (○). Entry of ^3H -PGF_{2 α} into the tissue resulted in some ^3H loss from M_o , but there was no evidence of ^3H -PGF_{2 α} entry into the lumen. In contrast, ^{14}C -thiourea, a substance which freely penetrates cell membranes, rapidly crossed the tissue and a thiourea equilibrium between M_o and M_i was approached within 2 h.

New Zealand white rabbits (2.1–3.3 kg) were killed with an overdose of Nembutal. The vagina was freed by blunt dissection and cut near the pelvic bone and just below the cervix. One end of the resulting tube was fitted with, and tied to, a Plexiglass tube and the other end was ligated to form a bladder-like preparation. The resulting sac was filled with 1 or 2 ml Earle's tissue culture medium (Microbiological Associates) and was lowered into a vessel containing 2 ml culture medium. The Plexiglass tube served as a reservoir for the inside medium during the periods of vaginal constrictions which typically lasted for 10 s with a periodicity of 0.5 to 3 min. To minimise variations in results, only vaginas which could be fitted over the 8 mm diameter tip of the Plexiglass tube and the walls of which were translucent, and less than about 2 mm thick in the relaxed state, were used. As the surface areas of these bladder-like preparations could not be accurately determined, individual rates could not be standardised to a unit surface area. Each figure represents, therefore, results obtained on one preparation, typical of a set of four or more similar experiments. The assembly was maintained in a bath at 37 °C or at 2–4 °C. In most experiments, trace amounts of ^{14}C -sucrose, ^{14}C -thiourea, ^{14}C -octanoic acid or ^{14}C -linoleic acid were also added to either M_o , M_i , or both, to test the passive permeability properties of the vagina and the specificity of this transport system. Samples (20 μl) of both M_o and M_i were taken at 5–20 min intervals, oxidised using a Packard Tricarb Model 306 automatic sample oxidiser, and the separate ^3H and ^{14}C samples counted for 10 min or 10,000 counts in a liquid scintillation counter. In some experiments both media were changed, after 60 or 90 min, to one containing a high concentration of unlabelled PG, or another potential inhibitor, in addition to the ^3H and ^{14}C -labelled compounds. After 30–50 min of further sampling, both inside and outside compartments were washed with, and replaced by, a medium containing a higher inhibitor concentration or with the original, inhibitor-free, radioactive medium.

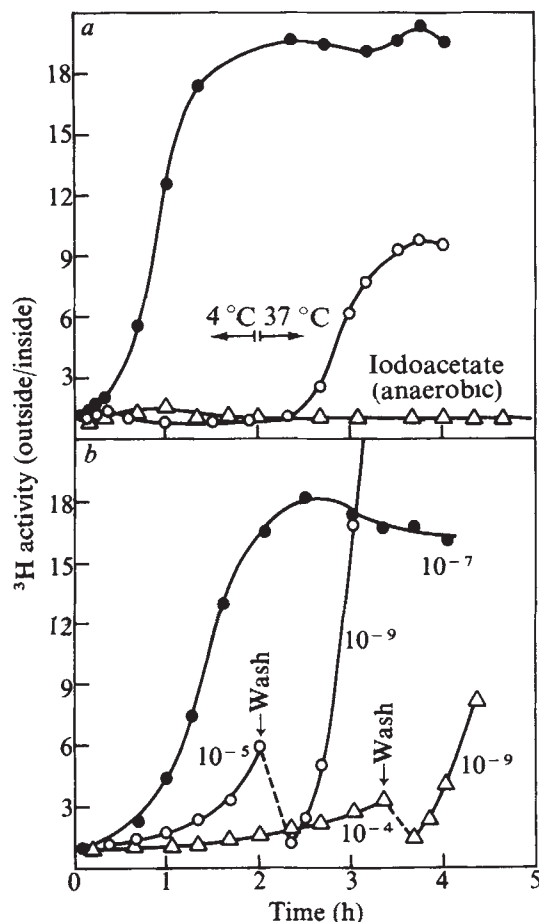


Fig. 2 Effects of temperature, metabolic inhibition and high $\text{PGF}_{2\alpha}$ concentrations on development of ^3H gradient across isolated rabbit vagina incubated with ^3H - $\text{PGF}_{2\alpha}$. a, When M_i and M_o had the same initial ^3H - $\text{PGF}_{2\alpha}$ activity (total $[\text{PGF}_{2\alpha}]$ approximately 10^{-9} M) the $M_i \rightarrow M_o$ flux established a 20-fold M_i/M_o ^3H gradient at 37°C (●), whereas at 4°C the M_i/M_o ratio remained at unity until the temperature was raised to 37°C (○). The ^3H ratio remained at unity indefinitely in anaerobic conditions (at 37°C) in the presence of 2 mM iodoacetate (△). b, The development of M_o/M_i ^3H gradient showed a dose-dependent, reversible inhibition when the $[\text{PGF}_{2\alpha}]$ in both M_o and M_i was raised from 10^{-7} (●) to 10^{-5} (○) or 10^{-4} M (△).

was slightly reduced, but the formation of 15-keto- $\text{PGF}_{2\alpha}$ was greatly suppressed; thus, in five experiments $89 \pm 2\%$ ^3H activity in M_o and $91 \pm 1\%$ in M_i remained associated with the $\text{PGF}_{2\alpha}$ spot on the A1 system¹⁴. In this condition, a net flux of chromatographically identifiable ^3H - $\text{PGF}_{2\alpha}$ against a concentration gradient was observed which resulted in a final, paper chromatographically identifiable, $\text{PGF}_{2\alpha}$ M_o/M_i ratio of 7.3 ± 2.5 ($n = 5$). The fact that in the presence of furosemide the bulk of the accumulated ^3H remained associated with $\text{PGF}_{2\alpha}$ was also substantiated in four such experiments with a previously described¹¹ immunoadsorption technique.

To provide further evidence that $\text{PGF}_{2\alpha}$ transport does not require a metabolic alteration of the PG molecule, five similar experiments were carried out in which both M_i and M_o contained 10^{-6} M $\text{PGF}_{2\alpha}$ and 10^{-4} M furosemide but no ^3H - $\text{PGF}_{2\alpha}$. After 2 h incubation, the fluids were removed and the PGs extracted¹³. Immunoassay of these extracts with an anti- $\text{PGF}_{2\alpha}$ serum which shows negligible cross-reactivity with 15-keto- $\text{PGF}_{2\alpha}$ (ref. 11), showed that the $\text{PGF}_{2\alpha}$ content of M_i decreased, whereas that of M_o increased, yielding more than fivefold final M_o/M_i $\text{PGF}_{2\alpha}$ gradient. Bioassay of three of these extracts on gerbil colon¹⁶ also showed that the $\text{PGF}_{2\alpha}$ -like activity of M_o increased whereas that of M_i decreased. As in all these experiments the initial $\text{PGF}_{2\alpha}$ concentration of

$M_i = M_o$, these results substantiate the isotope studies indicating that chemically unaltered, biologically active, $\text{PGF}_{2\alpha}$ can be transported across a biological membrane against a true concentration gradient.

In three similar experiments, $\text{PGF}_{2\alpha}$ was omitted from the initial incubation medium to see whether locally synthesised $\text{PGF}_{2\alpha}$ is released into M_o or M_i . After 2 h incubation, a small amount (0.6 ± 0.1 ng; $n = 3$) of $\text{PGF}_{2\alpha}$ was found by immunoassay in M_o only. This amount of PG release could not significantly affect the observed PG gradient, but indicates that PGs which are synthesised by the vagina are either released entirely on the serosal side of the barrier or are transported across this barrier preventing their accumulation in the lumen.

The existence of such active PG transport processes could have physiological significance, not only in the case of the vagina¹⁰, blood-ocular fluid⁸ and blood-brain⁹ barriers, but also with respect to the general distribution and metabolism of these autocooids⁷. PG accumulation against a concentration gradient by the kidney cortex¹, for example, suggests that excretion and/or renal distribution of PGs and/or their metabolites also depends on a carrier-mediated process. PG accumulation by lung tissue¹ and its saturable uptake into isolated perfused rat lung preparation¹⁷ suggests that a carrier-mediated uptake of PGs into the cells may be required for the rapid pulmonary metabolism of PGs.

In conclusion, the energy-dependent, saturable net flux of $\text{PGF}_{2\alpha}$ across the isolated rabbit vagina provides direct experimental evidence in support of the concept¹ that PGs can be actively transported by some vertebrate tissues. Concurrent *in vitro* studies on rabbit anterior uvea, choroid plexus and kidney cortex^{2,3} indicate that this PG transport mechanism is distinctly different from the iodide transport system, it overlaps with the *p*-aminohippurate system and may be related to the 'L' component of the iodipamide transport system which was described recently¹⁸. This PG transport system can be expected to have a fundamental role in the removal of PGs from the extracellular fluids of the eye⁸ and the brain⁹ and may also have a role in the pulmonary metabolism and renal excretion of PG (ref. 7). The existence of such PG transport processes implies that inhibitors of PG transport may provide an experimental and/or therapeutic tool for the manipulation of the distribution and disposition of PGs.

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Differential effects of cyclic AMP on the *in vitro* induction of antibody synthesis

INTRACELLULAR events in lymphocytes following antigenic stimulation still remain largely obscure. It has recently been demonstrated that cyclic AMP is involved during early events of the *in vitro* immune induction, since exogenous application of cyclic AMP can prevent spleen lymphocytes from differentiating into antibody-producing cells¹. One can assume that this is more than a general effect on cell division processes, since inhibition by cyclic AMP could be demonstrated only during the first 24 h of the immune induction period, whereas cell proliferation starts after 24 h (ref. 2). Since cooperation between T and B cells and proliferation as well as differentiation of these cells are essential features during the early period of the immune response against most antigens³ it was of interest to study the effect of cyclic AMP on the primary immune response in a T-cell-deficient system such as nude spleen cell cultures. Nude spleen cells can be stimulated to antibody production against sheep red blood cells (SRBC) not only in the presence of allogeneic T cells and antigen^{4,5} but also by B cell mitogens, such as lipopolysaccharides (LPS) from various bacteria without a specific antigen⁶.

Here, the effect of exogenous cyclic AMP on the T-cell-dependent immune response to SRBC is compared with the T-cell-independent activation by the mitogen LPS and possible events following treatment by cyclic AMP are discussed.

As shown in Table 1, nude spleen cells give rise to only small numbers of antibody-producing cells in the absence of allogeneic thymus cells or a potent mitogen like LPS.

and specific antigen (Table 1), whereas cultures reconstituted with thymus lymphocytes and stimulated by SRBC showed a reduction in the number of plaque-forming cells on day 4.

It was of interest to see whether cyclic AMP affects those events which occur during the cooperation of thymus-derived and bone marrow-derived lymphocytes or rather other processes which are involved in antigen recognition and T cell maturation. Therefore, antigen-activated T cells, as matured T cells, were used to restore the immunological capacity of nude spleen cells in place of allogeneic thymus cells. The effect of cyclic AMP in this culture system was tested and compared with cultures restored with normal allogeneic thymocytes from 4-week-old donors.

From the data of Table 2 we can see that the induction of antibody synthesis is suppressed by cyclic AMP only when normal allogeneic thymus lymphocytes are used for restoration. The addition of cyclic AMP to nude spleen cells restored with antigen-activated T cells did not lead to inhibition of day 4 antibody synthesis against SRBC. No difference in the insusceptibility to cyclic AMP was seen when donors of three different H-2 backgrounds were used as the source of activated T cells. This cooperation across the H₂-barrier may suggest parallels to allogeneic stimulation. In this case, one must conclude that the cyclic AMP independence of antigen-activated T cells in this respect indicates that they have reached a stage in development differing from normal allogeneic T cells.

Differences in the intracellular level of cyclic AMP have been described for different stages in differentiation of mammalian cells¹⁰. Since it was found that inhibition of the immune induction by cyclic AMP can be effected only during the first 24 h (ref. 1), it was of interest to see whether this intracellular mediator is involved in cooperation events during the early period of the immune induction or rather influences processes

Table 1 Influence of cyclic AMP on the immune response to SRBC in *nu/nu* spleen cells

Cell cultures	Exp. 1	PFC per 10 ⁶ collected cells Exp. 2	Exp. 3	Exp. 4	Exp. 5
<i>nu/nu</i> spleen cells (3 d)	79	—	60	68	55
<i>nu/nu</i> spleen cells (3 d)+SRBC	33	—	72	86	94
<i>nu/nu</i> spleen cells (3 d)+LPS	184	172	310	390	213
<i>nu/nu</i> spleen cells (3 d)+LPS+SRBC	203	142	—	—	—
<i>nu/nu</i> spleen cells (3 d)+LPS+cyclic AMP	164	231	309	295	264
<i>nu/nu</i> spleen cells (4 d)+SRBC	47	63	—	—	—
<i>nu/nu</i> spleen cells (4 d)+SRBC+Thy	303	299	—	—	—
<i>nu/nu</i> spleen cells (4 d)+SRBC+Thy +cyclic AMP	56	54	—	—	—

Cell cultures of spleen cells from *nu/nu* mice with NMRI background were prepared according to the method of Mishell and Dutton⁷. Sheep erythrocytes (SRBC) were used as antigen at a dose of $3 \times 10^6 - 5 \times 10^6$ SRBC per 10^7 cells. Culture dishes reconstituted with thymus lymphocytes contained 1×10^7 nude spleen cells and 5×10^6 thymus cells or 5×10^6 antigen-activated thymus cells, whereas cell cultures supplemented with LPS contained only 1.5×10^7 nude spleen cells. After 3 or 4 d of *in vitro* incubation five culture dishes of one group were pooled. The number of antibody-producing cells was determined with a modified Jerne plaque technique⁸. Cell viability was checked by Trypan blue dye exclusion tests. The number of plaque-forming cells was determined after two different times of incubation *in vitro*. Spleen cells stimulated with LPS were collected on day 3 and cell cultures restored by allogeneic spleen cells were pooled and assayed on day 4 of incubation. Lipopolysaccharides (LPS) from *Salmonella anatum* were used to stimulate nude spleen cell cultures. LPS was extracted by the phenol-water method and purified by ultracentrifugation⁹. It was dissolved in Eagle's medium to a final concentration of $10 \mu\text{g ml}^{-1}$. Thy, allogeneic thymus lymphocytes from 4-week-old C57BL; cyclic AMP was used at 10^{-3} mol.

On day 3 the number of plaque-forming cells in cultures to which SRBC had been added did not differ significantly from control cultures without antigen. On the other hand when LPS or allogeneic thymus lymphocytes were added, these nude spleen cell cultures produced increased numbers of plaque-forming cells on day 3 or day 4. There was no significant difference in the 3-d response between cultures supplemented with LPS alone or with both LPS and SRBC, when $10 \mu\text{g ml}^{-1}$ LPS was used.

Parallel cultures were treated with 10^{-3} mol cyclic AMP (Boehringer, Mannheim) at the beginning of incubation. This dose was shown to inhibit the immune induction of normal spleen cultures in previous experiments¹. The application of cyclic AMP has no inhibitory effect on nude spleen cell cultures activated polyclonally with LPS in the absence of thymus cells

dependent on antigenic interaction with T cells.

The data presented here lead to the conclusion that exogenous application of cyclic AMP probably affects those events in the immune induction which are related to T cell function, since LPS-induced differentiation of B cells to antibody-producing cells cannot be disturbed by exogenous cyclic AMP. Also experiments with antigen-activated T cells indicate that the interaction of T and B cells itself is not affected by cyclic AMP, since cooperation can occur in nude cell cultures restored by matured T cells in the presence of cyclic AMP. Because only one of the three culture conditions presented here was influenced by cyclic AMP (namely, nude spleen cells restored by naive thymus lymphocytes) one can conclude that exogenous cyclic AMP disturbs processes which lead to T cell maturation after antigenic stimulation.

Table 2 Influence of cyclic AMP on the immune response to SRBC in *nu/nu* spleen cells* restored with antigen-activated T cells

Cell cultures	Experiment 1†	PFC per 10 ⁶ collected cells Experiment 2‡	Experiment 3§
<i>nu/nu</i> spleen cells + SRBC	5	0	13
<i>nu/nu</i> spleen cells + Thy + SRBC	161	281	157
<i>nu/nu</i> spleen cells + t-act + SRBC	195	489	309
<i>nu/nu</i> spleen cells + Thy + SRBC + cyclic AMP	35	2	2
<i>nu/nu</i> spleen cells + t-act + SRBC + cyclic AMP	408	680	300

For the preparation of antigen-activated T cells 8–12-week-old mice were lethally irradiated with 750 r. They received $1 \times 10^7 - 3 \times 10^7$ thymus lymphocytes intravenously from a syngeneic donor, 4 weeks old. At the same time 3×10^8 SRBC were injected intraperitoneally into each recipient. After 6 d mice were killed, their spleen cells collected and used as antigen-activated T cells (t-act).

Thy, thymus lymphocytes from 4-week-old C57BL; PFC, plaque forming cells.

* *nu/nu* mice derived from the 10th backcross generation with BALB/c.

† C57 mice

‡ C3H mice

§ BALB/c mice } donors of antigen-activated T cells.

The differential effect of cyclic AMP on nude spleen cell cultures supplemented either with allogeneic thymus cells or antigen-activated T cells corresponds to the differential effect of cyclic AMP in normal spleen cultures, when the immune response is prevented only during a critical time during immune induction¹. Therefore it seems reasonable to relate the action of this cyclic nucleotide in normal cells to early processes in thymus lymphocytes induced by antigen. Although Watson *et al.* found that the immune response is inhibited when B cells are treated with cyclic AMP before antigen presentation¹², more recent results of Watson¹³ and others (J. Anderson, F. Melchers, and S. Bauminger, personal communication) demonstrate that the intracellular level of cyclic AMP in nude spleen cells is not changed significantly after stimulation by LPS, whereas the intracellular level of cyclic GMP rises dramatically.

Our results are also related to other reports on cyclic AMP binding sites on the cell surface of thymus lymphocytes and intracellular measurements which show changed levels of cyclic AMP in thymus lymphocytes¹¹.

Treatment of nude spleen cells with theophylline results in inhibition of antibody production after LPS stimulation (R. Bösing-Schneider, unpublished). This effect is not understood, because the intracellular level of cyclic AMP in these cells is not changed after stimulation by LPS (ref. 13). Since cyclic GMP seems to have an important role in these cells¹³ one can question if the cyclic GMP phosphodiesterase is affected by theophylline.

Our experiments have shown that exogenous cyclic AMP had an inhibitory influence only in cell cultures restored by allogeneic thymocytes and stimulated by antigen, whereas in cultures activated polyclonally⁶ with LPS, in the absence of thymus cells and antigen, no inhibition effect was detectable. Further experiments with antigen-activated T cells lead to the conclusion that treatment of cell cultures with cyclic AMP may affect only those antigen dependent processes which lead to T cell maturation.

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Evidence for the induction of two antibodies with identical combining sites in outbred animals

THE mechanism by which the immune system produces an apparently limitless array of antibodies in response to a variety of antigenic stimuli remains an unresolved biological question. Primary structural analysis has shown that immunoglobulin heavy (H) and light (L) chains have both variable (V) and constant (C) regions of amino acid sequence. Within the V_H and V_L region sequences there are areas of hypervariability which are thought to be associated with the antibody combining site. It is the amino acid substitutions within certain of these hypervariable areas that result in the multiplicity of antibody specificities (sites) expressed in nature. It seems clear that separate genes code for the V and C regions of H and L chains and that integration of V- and C-region genes occurs at the DNA level¹. One can account for antibody isotypic diversity by postulating a limited number of C-region genes which are transmitted from generation to generation in the germ line. The difficulty arises in the attempt to account for the apparent large numbers of V-region genes required to explain antibody combining site diversity. V-gene counting has been accomplished by RNA–DNA hybridisation^{2–4}. Evidence from these studies has not definitely demonstrated whether the capacity to generate large numbers of combining sites exclusively arises by either the inheritance of a complete set of V_H and V_L genes in the germ line or by somatic mutation. The repeated occurrence of V-gene products has, however, been indicated by sequence analysis of myeloma proteins^{5–7}, isoelectric focusing^{8–10}, and idiotype analysis^{11–13}.

It thus seems that an abundance of evidence favours repeated V-region genes in inbred systems and supports the germ line hypothesis^{14–16}. It now becomes important to map particular V regions and to show whether they are stably retained in an outbred population. Support for the stability of V-region genes in outbred systems comes from the discovery of apparently identical phosphorylcholine combining sites in different inbred mouse strains¹³ as well as highly similar V-region sequences (differing by only 8 residues, 3 being located in the hypervariable regions) in two human IgM myeloma heavy chains obtained from genetically distinct individuals¹⁷. To gain additional insight into V-region gene transmission and stability in a randomly outbred species we are currently mapping V regions from antibodies directed against the DNP determinant. Our studies have taken advantage of the antigen (DNP)₂-gramicidin S,

which reproducibly elicits antibodies of limited structural heterogeneity (refs 18, 19), thereby maximising recognition of particular V regions. Here we examine evidence suggesting that antibodies with identical combining sites have been elicited in two outbred rabbits and provide additional evidence that the genetic information necessary to account for V-region (combining site) diversity is present and stably transmitted in the germ line.

A detailed analysis of the immune response to (DNP)₂-gramicidin S has been reported previously^{18,19}. These studies showed that a number of rabbits producing anti-DNP antibodies possessed monoclonal characteristics¹⁹. Initial assessment of monoclonality was based on: (1) the spectral similarities of these antibodies to myeloma proteins and (2) the lack of binding heterogeneity as reflected in unitary heterogeneity indices. Further evidence for homogeneity was obtained from chain reassociation studies in which these purified antibodies regained their original binding constants after reduction, alkylation and exposure to dissociating conditions¹⁹.

Figure 1a shows the isoelectric spectra of six rabbits possessing monoclonal spectrotypes. It is evident that the monoclonal responses were not confined to rabbits possessing identical serum allotypes nor did individual antibodies necessarily possess identical spectrotypes. Two antibodies

Fig. 1 Serum aliquots (100 μ l) or purified antibodies (200–400 μ g) were focused in 5% thin-layer polyacrylamide gels containing pH 3.5–10 or 6–8 ampholine carrier ampholytes (LKB). Anti-DNP antibodies were seen after interaction with ¹²⁵I HDL, by contact autoradiography¹⁸. *a*, Isoelectric analysis of serum from rabbit 13 (track 1, allotype a13, b44), rabbit 22 (track 2, allotype a12, b44), rabbit 71 (track 3, allotype a13, b44), rabbit 126 (track 4, allotype a33, b44), rabbit 127 (track 5, allotype a33, b44) and rabbit 129 (track 6, allotype a13, b44) on pH 3.5–10 ampholyte gels. These characteristic monoclonal anti-DNP antibody spectra were perpetuated with antigenic stimulation until the death of each rabbit. *b*, Isoelectric analysis of antibodies from rabbits 13 (tracks 5 and 6; 6 and 18 months after initial immunisation) and 22 (1, 2 and 3; 6, 12 and 24 months after initial immunisation) on a pH 6–8 ampholyte gel. Track 4 was obtained by mixing equal quantities of purified antibodies from each rabbit.

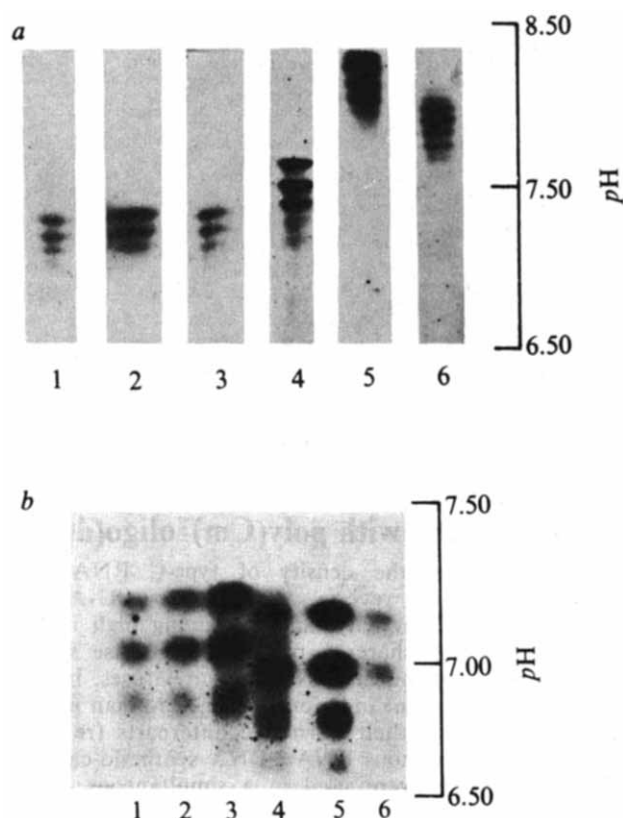


Table 1 Association constants of anti-DNP antibodies before and after recombination*

Sample	Mean K_0 (M^{-1})	
	Before	After
MOPC-315	1.2×10^6	1.3×10^6
anti-DNP-BGG (pool)	4.6×10^9	$(1.2 \times 10^6)^{\dagger}$
anti-DNP-GS		6.7×10^5
13	1.3×10^6	$(7.6 \times 10^5)^{\dagger}$
22	1.4×10^6	$(1.2 \times 10^6)^{\dagger}$
H13†	$< 10^5$	—
L13†	NA§	—
H22	$< 10^5$	—
L22	NA§	—
H13†+L22	—	1.2×10^6
H22+L13†	—	1.3×10^6
H22+L (Ab pool)¶	—	$< 10^5$

*Recoveries of 7S recombinants compared with the starting material ranged from 30 to 55%, the dialysis bag method averaging 5% higher recovery than the chain separation method. Recombinant recoveries for pooled anti-DNP-BGG antibodies and heterologous chains [H22+L (Ab pool)] ranged from 30–35%. Recombinant recoveries for MOPC-315, 13, 22, and 13 and 22 cross chain mixtures were comparable and ranged from 50 to 55%.

†() experiments using non-separated chains; dialysis bag method.

‡¹⁴C-labelled chains.

§No measurable activity.

¶Light chains obtained from pool of heterogeneous anti-DNP antibodies.

(track 1, rabbit 13, and track 2, rabbit 22) which seemed to display similar spectrotypes, were analysed further on isoelectric gels containing expanded range ampholytes. Figure 1b shows a detailed analysis of rabbits 22 (tracks 1, 2, 3,) and 13 (tracks 5 and 6). Track 4 was obtained by mixing equal quantities of these two antibodies and shows that the spectra are not, in fact, identical. In view of our subsequent findings, these slight spectral differences may result from biochemical modifications outside the combining site and perhaps outside the V region. In spite of spectral differences, both antibodies possess identical association constants (Table 1) which suggests possible combining site similarities.

To test this possibility we undertook a series of chain recombination experiments. Chain recombination has been used previously to assess the heterogeneity of mouse²⁰ and rabbit²¹ antibodies. Our preliminary recombination studies with rabbit 13 and 22 antibodies had shown that complete regain of binding activity could be obtained when chains from homologous antibodies were allowed to reassociate (Table 1)¹⁹, a behaviour characteristic of monoclonal antibodies^{20,21}. We used cross chain recombination studies to study the relationship of the combining site structure of these two antibodies. Fig. 2 outlines the experimental protocols used in this analysis. The quantities of monoclonal antibodies precluded the exclusive use of separated chains in every experiment, so initially it was important to compare the two recombination protocols. Table 1 summarises the data from these experiments. The K_0 values on the left were obtained before reduction and alkylation, the values on the right after reassociation following the indicated protocol. Mouse MOPC-315 IgA, a myeloma protein with reactivity for DNP, completely regained its original K_0 , whereas a heterogeneous population of rabbit anti-DNP antibodies showed a drop of 4 log units. These data confirmed that the recombination system can distinguish monoclonal and heterogeneous antibody populations and justified the use of either recombination protocol. The data on the two monoclonal antibodies show that recombinant 7S molecules prepared from either H (13) and L (22) or H (22) and L (13) displayed K_0 values identical to recombinants prepared with homologous H and L chains. Control experiments showed that H (13) or H (22) did not possess K_0 values of parent molecules, nor did recombinants prepared using these H chains and L chains from other anti-

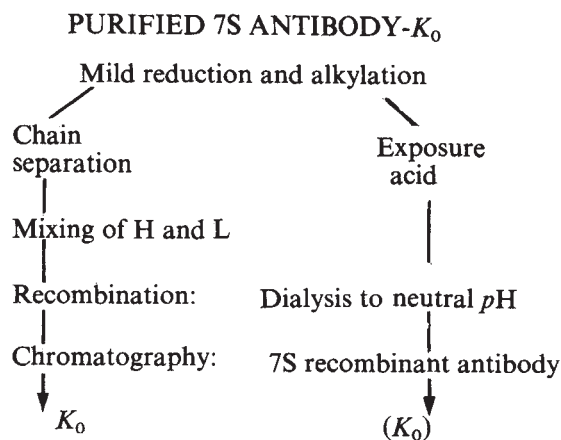


Fig. 2 Alternative protocols used in the recombination studies. Antibodies were purified by affinity chromatography on DNP-Sephadex immunoadsorbent columns¹⁹ and aggregates removed by chromatography & (Sephadex G-150). 7S antibodies were mildly reduced for 2 h in 0.1 M 2-mercaptoethanol in 0.2 M Tris (pH 8.0) under N_2 at 25 °C followed by alkylation for 30 min with a 10% molar excess of iodoacetamide (in certain cases $1\text{-}^{14}\text{C}$ iodoacetamide was used to label the H and L chains). The dissociation of the mildly reduced and alkylated chains was either accomplished by dialysis against 1 M propionic acid or by physical separation on Sephadex G-100 equilibrated in the same solvent. Separated chains (mixed in a ratio of 1.2 mol L chain per mol H chain) or dissociated chains were allowed to reassociate by sequential dialysis against 500-fold excess amounts of 0.05 M propionic acid (once), H_2O (twice) and borate buffered saline (pH 8.3) (twice). Recombinant 7S molecules were recovered from a calibrated Sephadex G-150 column¹⁹. Association constants (K_0) were determined by fluorescence quenching at 4 °C (ref. 19). Each K_0 value represents the average of at least three titrations.

DNP antibodies (Table 1 and unpublished observations). Jaton (Basel Institute for Immunology) has carried out recombination studies²² on homogeneous anti-pneumococcal type III antibodies which have been sequenced^{23,29}. Recombinant molecules prepared with H chains and heterologous L chains differing by as few as 6 hypervariable residues (total of 8 substitutions within the amino terminal 139 residues) do not regain appreciable binding activity, whereas recombinants prepared with homologous H and L chains do²². In fact, to date, Jaton (personal communication) and others²⁵ have only been able to achieve the binding activity of the parent molecule when H and L chains come from the same parent molecule. Our argument for combining site identity would, of course, be strengthened by amino acid sequence data. Jaton's findings demonstrate, however, that the chain recombination technique is a powerful probe of combining site fine structure²². The interpretation of our data in the light of these findings leads to the suggestion that rabbits 13 and 22 most probably possess identical combining sites.

The probability of two such antibodies arising purely by somatic mutations and being expressed in 2 out of as few as 100 rabbits seems low. Confidence in the random breeding of these two rabbits comes from the knowledge that each was immunised in a different country. The stability of these responses is illustrated by the fact that they were potentiated up to 1.5 (rabbit 13) to 2 (rabbit 22) years, without variation. When stimulation was discontinued for 6 months (rabbit 13), the response disappeared. Restimulation induced identical monoclonal antibody. Recent studies have suggested that two or more genes may code for heavy chain V regions, one specifying framework residues including the group a allotype, another specifying the idiotype^{26,27}. The two rabbits studied in detail here (rabbits 13 and 22) were heterozygous for the a allotype, both sharing the

a1 allotype. Finding the same allotype on antibodies with identical combining sites would suggest repetition of the entire V region, although sequence data would be needed to confirm such a speculation. It remains possible that a given combining site is not confined to a particular allotype and present investigations are directed at determining if identical sites may be present on antibodies of different allotypes.

The apparent ability to induce identical combining sites in outbred members of the same species lends strong additional support to the notion that a particular V-region gene may persist and be transmitted stably in the germ line while apparently undergoing minimal mutations in regions not affecting the combining site.

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Detection of reverse transcriptase in human breast tumours with poly(Cm)·oligo(dG)

PARTICLES having the density of type-C RNA tumour viruses, possessing reverse transcriptase (RNA-directed DNA polymerase) activity, and containing high molecular weight RNA that shares a portion of its base sequences with the RNA of murine RNA tumour viruses, have been reported to be present in several types of human malignant tissues, but not in their normal counterparts (review, see ref. 1). The endogenous RNA→DNA synthetic capabilities of these particles were used in a simultaneous detection test^{2,3} to determine these parameters. Because of their

similarity and relatedness to animal oncornaviruses, these particles are considered to be possible aetiological agents of human cancer².

To establish more firmly the presence of particles containing reverse transcriptase activity in human tumours, and to delineate the difficulties involved in the use of an exogenous synthetic probe to detect the enzyme in crude extracts, we have examined a number of human tissues for reverse transcriptase activity using poly(2'-*o*-methylcytidylate)-oligodeoxyguanylate [poly(Cm)-oligo(dG)] as template primer. Poly(Cm)-oligo(dG) is an effective template primer for every reverse transcriptase so far tested, including the enzymes from RNA tumour viruses of avian, murine, feline, and primate origin⁴ (G.F.G., unpublished). Neither bacterial DNA polymerase I nor any of the eukaryotic DNA polymerases tested, including the three major DNA polymerase species from mouse 3T6 or human KB and HeLa cells^{1,4,5}, can copy poly(Cm)-oligo(dG). In particular, highly purified DNA polymerase γ from HeLa cells, an enzyme that resembles viral reverse transcriptase in its ability to copy poly(C)-oligo(dG)⁶, does not copy poly(Cm)-oligo(dG)⁵. Poly(Cm)-oligo(dG) is therefore a specific template primer for viral reverse transcriptase. Using this specific probe, we have detected reverse transcriptase activity in particulate material isolated from the postmitochondrial cytoplasm of approximately 50% of the

human mammary tumours we have examined, but not in the cytoplasm of non-malignant breast tissue. It was necessary to remove interfering deoxyribonucleotide phosphatase activity present in human tissue extracts by phosphocellulose chromatography before reverse transcriptase activity could be detected.

We have taken the approach of isolating particulate material with a density between 1.15 and 1.20 g cm⁻³, a density range characteristic of RNA tumour viruses, from the postmitochondrial cytoplasm of human tissue, and after treating the particulate material with non-ionic detergent, assaying for DNA polymerase activity with poly(Cm)-oligo(dG) (for experimental details, see the legend to Table 1). This approach minimises interference in the assay by cellular enzymes, such as nucleases and other polymerases^{2,3}. It was discovered, however, that all tissue extracts examined, regardless of the human organ of origin, contained an inhibitor of poly(Cm)-oligo(dG)-directed poly(dG) synthesis, deoxyribonucleotide phosphatase (Fig. 1). By paper chromatographic analysis, the phosphatase activity was found to hydrolyse ³H-dGTP or ³H-TTP through mono and diphosphate intermediates to the deoxyribonucleoside level (G.F.G., unpublished). To facilitate removal of phosphatase from the cytoplasmic particulate fraction of tissues, we developed a rapid and convenient assay for dGTP phosphatase that uses residual ³H-dGTP in an activated calf thymus DNA-directed polymerising reaction catalysed by excess DNA polymerase (G.F.G. and P.M.L., in preparation). Details are described in the legend to Fig. 1.

Using this assay, we found that the postmitochondrial, cytoplasmic particulate fraction from 10 g of human liver, spleen, kidney, or breast that banded in the 1.15 to 1.20 g cm⁻³ density region of 15–65% sucrose gradients, contained sufficient phosphatase to hydrolyse from 0.8 to 1.2 μ mol of ³H-dGTP in 30 min. To carry out a poly(Cm)-oligo(dG)-directed assay in standard conditions without inhibition by phosphatase, the level of the enzyme in particulate cytoplasmic extracts would have to be reduced by 500 to 1,000-fold. The only technique we have found which removes enough phosphatase activity is phosphocellulose chromatography. In the presence of 0.1 M NaCl, phosphatase activity is not adsorbed by phosphocellulose, whereas reverse transcriptase from particles treated with Nonidet P-40 (NP-40) is bound and can be eluted with 0.25 M NaCl. By seeding human tissues (liver, kidney, spleen, breast) with various concentrations of RNA tumour viruses such as murine sarcoma-leukaemia or RD114 virus and using the procedure described in the legend to Table 1 to isolate reverse transcriptase, we have found in general that from 14 to 38% of the reverse transcriptase activity responding to poly(Cm)-oligo(dG) present in seeded virus is recovered in a volume of 2 ml from phosphocellulose columns.

Table 1 summarises our results with 19 human breast carcinomas and 9 non-malignant control tissues. Phosphocellulose eluates were assayed for DNA polymerase activity with poly(Cm)-oligo(dG), for terminal deoxyribonucleotidyl transferase activity with oligo(dG)⁷, and for background radioactivity without template or primer. Backgrounds averaging 235 $\pm$ 72 c.p.m. were subtracted in all cases. No significant transferase activity was detected in any of the tissue samples examined. The average incorporation observed with the non-malignant control tissues with poly(Cm)-oligo(dG) was 59 c.p.m. In contrast, the average with the malignant tissues was 837 c.p.m. On the basis of the magnitude and variability of the background trapping of radioactivity, there was a 90% probability that all tissues which gave 200 c.p.m. or more incorporation above background were positive for activity. On this evaluation, 53% of the malignant samples were positive for reverse transcriptase by the poly(Cm)-oligo(dG) assay and all the control samples were negative. It should be pointed out that 5 of

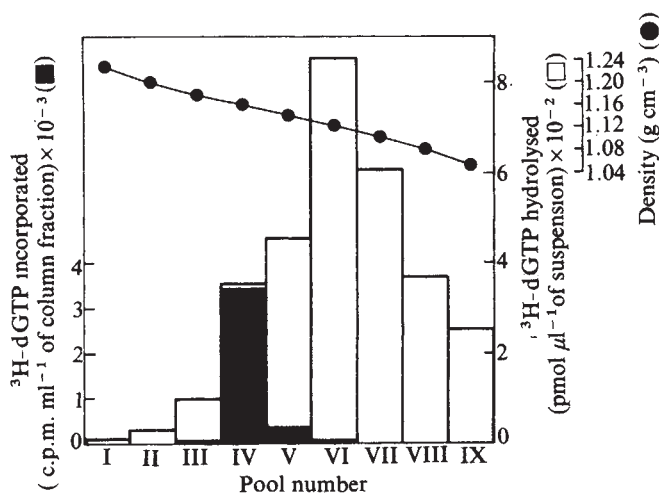


Fig. 1 Identification of the density of reverse transcriptase and phosphatase activity in a postmitochondrial extract of human mammary tumour by sucrose gradient centrifugation. A P-90 pellet from 13 g of human mammary tumour was prepared and fractionated on a 15–65% sucrose gradient as described in the legend to Table 1. Individual fractions within nine density regions were pooled, particulate material pelleted, and the pellets suspended as in Table 1. Samples (10 μ l) from each suspension were diluted and assayed for dGTP phosphatase (open bars) as follows. The phosphatase sample was added to a reaction mixture (50 μ l) containing 20 mM Tris-HCl (pH 7.8), 0.2 mM MnCl₂, and 3.7 μ M ³H-dGTP (2,900 c.p.m. pmol⁻¹). After 30 min at 37 °C, the reaction was terminated by heating at 100 °C for 2 min. The amount of ³H-dGTP remaining in the reaction mixture was then determined by using it as a substrate for excess DNA polymerase. A solution (50 μ l) was added to the heated mixture containing 10 mM Tris-HCl (pH 7.8), 10 mM dithiothreitol, 5 mM MgCl₂, 0.1 mM each dCTP, dATP, and dTTP, 300 μ g ml⁻¹ activated calf thymus DNA, and 1.4 units of *Micrococcus luteus* DNA polymerase (P-L Biochemicals). After a 60 min incubation at 37 °C, the amount of labelled DNA product formed was determined on Whatman DE-81 filters⁴. The total remaining suspensions (990 μ l) from density regions III (1.185–1.170 g cm⁻³), IV (1.165–1.150 g cm⁻³), V (1.145–1.130 g cm⁻³), and VI (1.125–1.115 g cm⁻³), were lysed, fractionated on separate phosphocellulose columns, and assayed for reverse transcriptase (solid bars) and transferase as in the legend to Table 1. Density regions I and II were directly assayed for reverse transcriptase activity after lysis without fractionation on phosphocellulose. No activity was observed in I and II. No transferase activity was observed in any density region.

the 9 malignant tissues which were negative weighed less than 5 g, which may have had a direct bearing on the low level of enzyme activity observed.

In addition to activity with poly(Cm)-oligo(dG), we have also examined phosphocellulose eluates prepared from a number of human carcinomas and non-malignant tissues for DNA polymerase activity with poly(C)-oligo(dG) and poly(A)-oligo(dT) in the presence of $MnCl_2$. Tissues negative for activity with poly(Cm)-oligo(dG) were also negative with poly(C)-oligo(dG), and positive responses to these template

primers were comparable in magnitude. In contrast, there was not always a correlation between response to poly(Cm)-oligo(dG) and to poly(A)-oligo(dT). For example, in both normal and malignant breast tissue extracts in which there was no response to poly(Cm)-oligo(dG), we sometimes observed no activity with poly(A)-oligo(dT) or activity which catalysed the incorporation of from 700 to 20,000 c.p.m. of 3H -TTP in response to poly(A)-oligo(dT). This activity probably represents variable contamination by DNA polymerase γ (ref. 6) of tissue extracts prepared by

Table 1 Detection of reverse transcriptase activity in breast tissue by poly(2'-*o*-methylcytidylate)-oligodeoxyguanylate

Breast tissue description			Incorporation of ^3H -dGTP (c.p.m. above background per 60 min incubation)		Designation
Type	Donor no.	Weight (g)	with		
Malignant			Poly(Cm) · oligo(dG)	Oligo(dG)	
Comedoadenocarcinoma	P-329	6.7	798	ND†	+
			415* 747†	ND	
Carcinoma	P-284	10.8	4,065	83	+
Infiltrative ductal adenocarcinoma	R-277A	8.7	314	-29	+
Adenocarcinoma	R-295A	10.2	2,079	16	+
			847*	ND	
Infiltrative ductal adenocarcinoma	R-291A	13.8	1,493	-22	+
			732*	-45	
Carcinoma	P-286	5.0	3,124	42	+
			2,846*	24	
Infiltrative ductal carcinoma	R-307A	5.4	962	18	+
Carcinoma	P-101	7.3	220	122	+
Infiltrative ductal adenocarcinoma	P-230	5.8	185	ND	+
			208*	ND	
Carcinoma	P-80	10	2,289	ND	+
			2,463*	ND	
Carcinoma	D-686C	11.9	1	ND	—
Carcinoma simplex	B-213A	6.2	86	-28	—
Carcinoma	P-241	3.6	34	12	—
Papillary carcinoma	R-204B	2.0	-8	6	—
Comedocarcinoma	D-662A	3.3	47	-67	—
Infiltrative ductal carcinoma	R-202C	5.7	-9	0	—
Carcinoma	D-797A	9.7	68	32	—
Infiltrative ductal adenocarcinoma	P-486	3.8	85	95	—
Infiltrative ductal adenocarcinoma	P-670	4.3	74	54	—
Non-malignant					
Normal	74-368	11.8	0	10	—
Normal	74-353	11.5	-25	-37	—
Normal	SM-3	3.9	-34	23	—
Fibrocystic	SM-1	9.9	84	29	—
Fibrocystic	P-636	8.8	129	5	—
Fibrocystic	P-644	13.9	53	49	—
Fibrocystic	P-649	8.3	116	4	—
Fibrocystic	P-651	5.8	115	91	—
Fibromastitis	P-635	5.1	93	3	—

Frozen human breast tissue, 2–14 g, was finely minced and disrupted with a Silverson homogeniser at 4 °C in 3 volumes of 0.01 M Tris-HCl (pH 8.3) + 0.15 M NaCl + 0.002 M EDTA (TNE)³. The suspension was centrifuged at 4,000g for 20 min at 4 °C and the pellet washed with 1 volume of TNE and recentrifuged. The supernatants were combined and centrifuged again at 10,000g for 10 min. The pellet was washed with TNE and recentrifuged. The 10,000g supernatants were combined and treated with trypsin (1 mg ml⁻¹ for 30 min at 37 °C) which was inactivated with Lima bean trypsin inhibitor (0.4 mg ml⁻¹). The treated supernatant was layered on a 12-ml column of 20% glycerol in TNE and spun at 90,000g for 1 h at 4 °C in an SW27 rotor. The resulting pellet (P-90) was suspended in 4 ml of TNE and layered on a 30 ml 15–65% (w/v) sucrose gradient in TNE and centrifuged at 90,000g for 12–14 h in an SW27 rotor. Fractions (40 to 50) were collected from below, and those in the density range of 1.145 to 1.20 g cm⁻³ were combined, diluted with TNE to bring the sucrose concentration below 10%, and centrifuged at 150,000g for 1 h in a Ti60 rotor. The resulting pellet was suspended in 1 ml of 0.02 M Tris-HCl (pH 7.8) + 0.001 M dithiothreitol + 0.1 mM EDTA + 10% glycerol (Buffer A), NaCl and NP-40 were added to 0.1 M and 2%, and the lysate incubated at 0 °C for 15 min. The lysate was applied to a 0.9 × 3 cm phosphocellulose (P-11, Whatman) column equilibrated in Buffer A + 0.2% NP-40. The column was washed with 20 ml of Buffer A + 0.2% NP-40 + 0.1 M NaCl, and eluted with 10 ml of Buffer A + 0.2% NP-40 + 0.8 M NaCl. Fractions of 1 ml were collected. A sample (250 µl) of the first fraction from the phosphocellulose column containing an increased concentration of NaCl (usually 0.15 to 0.2 M) was incubated for 1 h at 37 °C in a reaction mixture (500 µl) containing 20 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, 0.2 mM $MnCl_2$, 30 µM poly(Cm), 15 µM (dG)₁₂₋₁₈, and 2–4 µM 3H -dGTP ($2.3-2.7 \times 10^4$ c.p.m. pmol⁻¹). Additional samples were assayed (i) in the absence of poly(Cm) and (ii) in the absence of both poly(Cm) and (dG)₁₂₋₁₈. The amount of trichloroacetic acid insoluble radioactivity in the reaction mixture was determined by collection of the product on nitrocellulose filters after washing by repeated precipitation and dissolution with perchloric acid and NaOH⁴.

* A repeat 1-h incubation was carried out.

† A repeat 2-h incubation was carried out.

‡ Not determined.

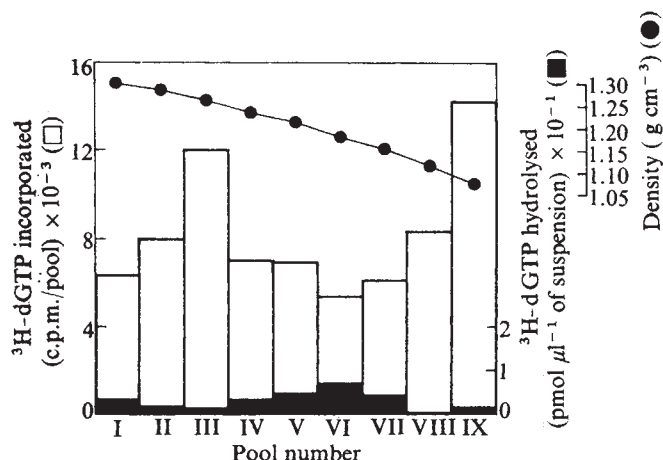


Fig. 2 Identification of the density of reverse transcriptase and phosphatase activity in an extract of human mammary tumour treated with nonionic detergent. A P-90 pellet from 50 g of human mammary tumour was prepared and fractionated on five, 15–65% sucrose gradients and particulate material with a density of 1.15 to 1.20 g cm^{-3} was pooled and pelleted in a Ti60 rotor as described in the legend to Table 1. The pelleted material was suspended in 600 μl of 0.1 M Tris-HCl (pH 8.3), and dithiothreitol and Sterox-SL were added to final concentrations of 100 mM and 1%, respectively. The lysate was layered on a 11.5-ml gradient of 25–85% (w/v) in TNE and was centrifuged at 180,000 g for 14 h in a SW41 rotor. The gradient was fractionated into nine density fractions, and the fractions were diluted with TNE to bring the sucrose concentration below 10% and were centrifuged at 150,000 g for 1 h in a Ti60 rotor. The resulting pellets were suspended in 200 μl of 0.01 M Tris-HCl, 0.01 M dithiothreitol, and 0.5% NP-40. Samples (10 μl) from each lysate were assayed for phosphatase (solid bars) as described in the legend to Fig. 1. Samples (50 μl) were directly assayed for reverse transcriptase activity (open bars) and transferase activity in 100 μl reaction mixtures as in Table 1. The average background incorporation was 225 c.p.m., and no appreciable transferase activity was observed in any fraction.

our procedures. The relative template efficiencies of poly(C)-oligo(dG), poly(A)-oligo(dT) and poly(Cm)-oligo(dG) with MnCl_2 for a purified mammalian RNA tumour virus (murine leukaemia virus) reverse transcriptase are 1.0, 0.7 and 0.2, respectively (refs 4 and 5 and G.F.G., unpublished).

To demonstrate that the DNA polymerase activity responding to poly(Cm)-oligo(dG) observed in human mammary tumours, but not in non-malignant breast tissue, is derived from RNA tumour virus-like particles, the DNA polymerase-containing material was further characterised with respect to two properties. First, the exact density of the particulate, poly(Cm)-oligo(dG)-directed DNA polymerase activity from a human breast tumour was determined by sucrose gradient equilibrium centrifugation. Figure 1 shows that the majority of material which contained DNA polymerase activity responding to poly(Cm)-oligo(dG) banded between 1.15 and 1.165 g cm^{-3} , a density range characteristic of RNA tumour viruses. Figure 1 also illustrates the large amount of phosphatase activity present in the cytoplasm of human breast tissue which peaks at a density of 1.11 to 1.13 g cm^{-3} in sucrose gradients, but overlaps into the 1.16 g cm^{-3} region.

Second, nucleoids or cores that contain the reverse transcriptase and possess a density of 1.26–1.27 g cm^{-3} can be generated from RNA tumour viruses by treatment of virions with non-ionic detergent⁸. By treatment with 1% Sterox-SL of the particulate material with a density of 1.15–1.20 g cm^{-3} from the postmitochondrial supernatant of 50 g of human mammary tumour, we were able to isolate reverse transcriptase activity responding to poly(Cm)-oligo(dG) that banded in the density range of 1.21 to 1.30 g cm^{-3} (peaked at 1.265 g cm^{-3}) on a 25–85% (w/v) sucrose gradient (see Fig. 2). This material probably repre-

sents cores of breast particles containing reverse transcriptase. DNA polymerase activity at the top of the gradient represents solubilised enzyme partially dissociated from particulate material and not contained within an intact core^{8,9}. Also demonstrated in Fig. 2 is the fact that most of the phosphatase contamination of the particle preparation (seen in Fig. 1) is eliminated by detergent treatment and subsequent equilibrium and velocity centrifugation, so that phosphocellulose chromatography before assay of DNA polymerase activity was not necessary.

The results reported here demonstrate that some human breast tumours, but not non-malignant breast tissues, contain particulate, poly(Cm)-oligo(dG)-directed DNA polymerase activity present in a structure possessing the density of an RNA tumour virus. Some of the DNA polymerase activity can be made to band at a density characteristic of virus nucleoids by treatment with a neutral detergent. In the light of these findings and the specificity of poly(Cm)-oligo(dG) for viral reverse transcriptase in other systems^{1,4,5}, it seems reasonable to conclude that we are detecting the enzyme in human breast tumours. It is clear from our experiment that the problem of phosphatase contamination is serious and must be overcome for successful demonstration of the enzyme. The synthetic probe provides an alternative to simultaneous detection³ for the identification of RNA-directed DNA polymerase activity in human tissues, and investigations are under way using poly(Cm)-oligo(dG) to search for the enzyme in other human tumours and differentiating tissues.

The significance of particle bound RNA-directed DNA polymerase in human breast cancers is not known. It is conceivable that these particles represent infectious, exogenous aetiological agents of the disease. An alternative hypothesis is that the cancer cell represents a partially differentiated cell that is blocked in further development and continues to express information specific for its developmental state, which could include RNA-directed DNA polymerase associated with specific RNA molecules¹. The retention of this information, normal for a specific state of development, would then be associated with the specific cancer cell type, and its expression could even be responsible for the malignant state of the cell.

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Polyadenylic acid sequences in *E. coli* messenger RNA

It is commonly assumed that the messenger RNA (mRNA) of prokaryotes lacks the poly(A) sequences which characterise the 3' terminus of most mRNA of eukaryotes. A report of the absence of poly(A) sequences from both pulse labelled and stable RNA of *Escherichia coli*¹ is often cited as evidence for such a distinction. This apparent difference has generated speculations ranging from the role of poly(A) sequences in regulating the longevity of eukaryotic mRNA molecules¹, to the evolutionary origin of mitochondria² in which poly(A) sequences have been found in a messenger-like RNA³⁻⁵. Such speculations prompted us to re-examine evidence for AMP-rich sequences in *E. coli* reported⁶ before poly(A) sequences were known to be attached to mRNA in animal cells⁷⁻⁹. We have found that RNA molecules can be isolated from *E. coli* with the techniques developed for the isolation of poly(A) containing RNA of eukaryotes¹⁰ which have properties of mRNA and contain poly(A) sequence at their 3' terminus.

Table 1 presents evidence for the presence of poly(A)-containing RNA in *E. coli*. A fraction of total RNA labelled either with a pulse of ³H-adenosine or for 10 min with ³²PO₄ could be bound to oligo(dT) cellulose. Seventy to eighty-five per cent of this RNA could be rebound to oligo(dT) cellulose, showing that nonspecific binding of RNA accounted for little of the RNA bound initially. These data compare favourably with those obtained from HeLa cell mRNA using the same conditions (Table 1, experiment 5). The poly(A) sequences released from these RNA fractions by treatment with ribonucleases and DNase contained about 15% of the total incorporated label showing that the fraction binding to oligo(dT) cellulose (Table 1) was neither free poly(A) nor merely an AMP-rich fragment.

In the ³H-adenosine pulse labelling experiments, the fraction of the total RNA binding to oligo(dT) cellulose was considerably lower than would be expected if all mRNA species contained a poly(A) sequence of at least 20 nucleotides which is close to the lower limit of poly(A) length that can be isolated in our conditions (unpublished). In view of the rapid turnover of *E. coli* mRNA and the relatively small size of the poly(A) sequence, variations in the fraction of RNA binding to oligo(dT) may be expected. The average size of poly(A)-containing RNA, as well as the size of the poly(A) sequence itself, tended to vary in different experiments (data not shown). The discrepancy in the fraction of poly(A)-containing RNA binding to oligo(dT) cellulose in the two ³²P-labelling experiments could be explained by the fact that the average lengths of the RNA and its poly(A) sequence in experiment 6 were considerably shorter than in experiment 7 (data not shown).

A rapid turnover of either the poly(A)-containing RNA or the poly(A) sequence is suggested because the poly(A)-containing RNA binding to oligo(dT) cellulose comprised much less of the total stable RNA species of *E. coli* (experiment 4, Table 1).

Figure 1 shows the electrophoretic mobilities of both bound and unbound RNAs in denaturing polyacrylamide gels (98% formamide), as well as the poly(A) sequence released by RNases from the bound RNAs. As Fig. 1b shows, bound RNA migrated more slowly than tRNA and almost no label migrated with a mobility similar to the poly(A) derived from this same RNA fraction. Clearly the failure to find label in gel fractions migrating in the region of the poly(A) sequences means that poly(A) must be covalently attached, since this RNA had been denatured by heating at 60 °C in a large excess of unlabelled poly(A) before its application to denaturing gels. Any measurable labelled poly(A) hybridised to RNA should have been lost through competition with the excess of unlabelled poly(A) present during denaturation.

Figure 1b shows that poly(A)-containing RNAs are poly-

Table 1 Isolation of poly(A)-containing RNA from *E. coli*

Experiment	Labelling time (min)	First binding to oligo(dT) cellulose (% total RNA)	Second binding to oligo(dT) cellulose (% first bound RNA)	Poly(A) content (% second bound RNA)
a ³ H-adenosine				
1	0.5	0.23	87.9	17.7
2	1.0	0.25	81.4	17.7
3	1.5	0.14	76.1	12.6
4*	120.0	0.007	68.1	—
5†	90.0	15.8	93.6	22.4
b ³² PO ₄				
6	10.0	0.16	71.2	17.7
7	10.0	1.41	86.0	11.5

E. coli D 10 (ref. 11) (supplied by Dr D. Nakada) grown in modified M9 minimal medium¹² supplemented with vitamin B₁ (2.5 µg ml⁻¹), glucose (0.4%) and casamino acids (0.4%) were collected at a density of 1 × 10⁸ cells per ml and were suspended in fresh growth medium at a concentration of 2 × 10⁸ cells per ml and vigorously aerated for 10 min before addition of ³H-adenosine (25–50 µCi ml⁻¹ at 31 µCi mmol⁻¹) for the time specified. For ³²P labelling, cells recovered from growth medium were washed twice with phosphate-free medium¹³ supplemented with the 20 L-amino acids, each present at 0.5 mM. After resuspending in growth medium, they were aerated for 10 min as above before the addition of ³²PO₄ (carrier free, 250–500 µCi ml⁻¹) for the specified time. Cells were lysed by the procedure of Okamoto *et al.*¹⁴. Unlabelled poly(A) (60 µg per 8 × 10¹⁰ cells) was added before the cells were rapidly frozen in lysing medium at –70 °C. The mixture was thawed and rapidly frozen three times before the addition of 1/10 volume of 0.5 M sodium acetate buffer (pH 5.2) containing 0.1 M EDTA and 1/40 volume of 20% sodium dodecyl sulphate (SDS). RNA was extracted from the lysate by three successive phenol extractions at 60 °C as described by Girard¹⁵. This RNA was dissolved in 3.0 ml of NETS (0.01 M Tris-HCl, pH 7.2; 0.1 M NaCl; 0.01 M EDTA and 0.5% SDS), and was mixed with 500 mg of oligo(dT) cellulose suspended in 2 ml of NETS. The mixture was heated for 3 min at 60 °C, before shaking for 60 min in a water bath at 23 °C. The RNA binding to oligo(dT) cellulose was recovered at low ionic strength as described¹⁰ before the addition of 150 µg of RNase-free soluble yeast RNA and NaCl to 0.1 M and 2.5 volumes of ethanol. The precipitate recovered after 16 h at –20 °C by centrifugation for 45 min at 20,000g was dissolved in NETS and was rebound and eluted from oligo(dT) cellulose. The bound fraction recovered after this second binding was precipitated as above. It was dissolved in 1.7 ml of 30 mM Tris-HCl, pH 7.4, containing 100 units of RNase T₁ and 0.1 µg RNase A (ref. 7). After 15 min at 37 °C it was cooled to 0 °C before adding 0.1 ml of 1 M Tris, pH 7.4, 0.16 ml of 0.1 M MgCl₂ and 0.02 ml of 0.2 M CaCl₂ and 10 µg of DNase. After 15 min at 37 °C the sample was applied to oligo(dT) and the poly(A) sequence was recovered as described¹⁶.

*18 × 10⁸ cells in 25 ml of medium were labelled with 1 mCi of ³H-adenosine at 80 µM.

†HeLa cell cytoplasmic RNA was isolated after labelling cells with 0.5 mCi of ³H-adenosine as described previously¹⁶.

Table 2 Nucleotide composition of poly(A) sequence

Treatment	Total poly(A) (c.p.m.)	CMP (%)	AMP (%)	GMP (%)	UPM (%)	Recovery (% total poly(A))
RNase T ₁	71,000	6.7	82.3	0.7	10.2	100
RNase T ₁ + RNase A	46,000	2.1	95.6	1.6	0.7	92

Poly(A) was isolated from a poly(A)-containing RNA labelled for 10 min with ^{32}P . Nuclease treatment as described in Table 1 except that in one case RNase A was omitted. After gel electrophoresis, fractions were pooled as shown in Fig. 2, and the poly(A) recovered was hydrolysed with 0.3 N KOH for 18 h at 37 °C. Nucleotide composition was determined by the method of Osawa *et al.*¹⁹.

disperse, migrating from 4S to 20S. When the logarithm of molecular weight of HeLa 28S, 18S and tRNA which were coelectrophoresed with this RNA and the poly(A), were plotted against their relative mobilities¹⁸, molecular weights of 130,000 and 13,000 were calculated for peak positions of poly(A)-containing RNA and poly(A) respectively, which agreed well with the 11.5% poly(A) content of this RNA.

The polydispersity and greater concentration of these poly(A)-containing RNAs in pulse-labelled RNA suggest

they are species of mRNA although evidence for their translation has not been sought. A relatively small fraction of the total label in the unbound RNA was in ribosomal RNA in these conditions (Fig. 1a), showing that labelled mRNA comprised a significant amount of total RNA. If at least

Fig. 1 Electrophoresis of poly(A)-containing RNA and poly(A) in polyacrylamide gels in formamide. Aliquots of RNA and poly(A) from experiment 7 in Table 1 were mixed with 20 µg of non-radioactive poly(A) and ^3H -labelled HeLa cell RNA in 98% formamide containing 20 mM NaCl and 10 mM Tris HCl, pH 7.6, and heated for 1 min at 60 °C. After cooling at 0 °C, bromophenol blue and sucrose were added. Electrophoresis in 19 cm of 4.2% polyacrylamide gels in formamide was carried out for 5 h at 11 V cm⁻¹ as described¹⁸. Fractions collected with a Maizel fractionator were counted in a toluene scintillation fluid containing Triton X-100. *a*, Unbound RNA (37,000 cm); *b*, bound RNA (46,000 c.p.m., ○) and poly(A) (5,300 c.p.m., ●) electrophoresed on parallel gels are plotted on the same figure. — dye marker; ↓ HeLa 28S RNA (molecular weight 1.9×10^6), 18S RNA (0.71×10^6) and 4S RNA (27,000).

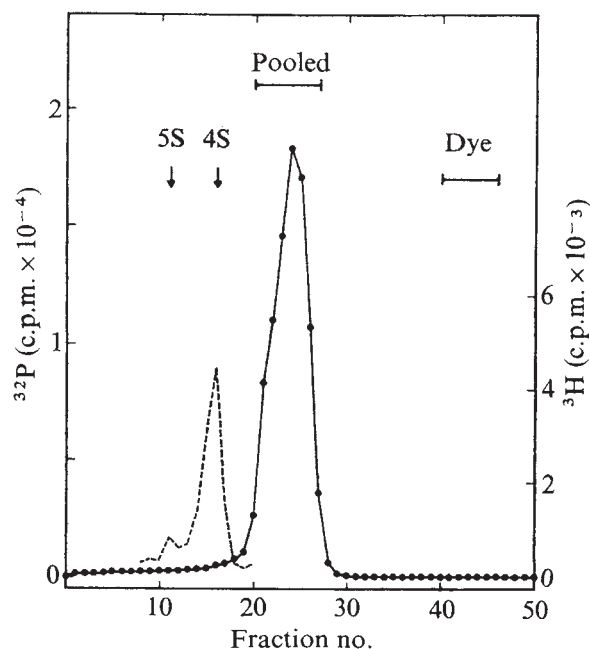
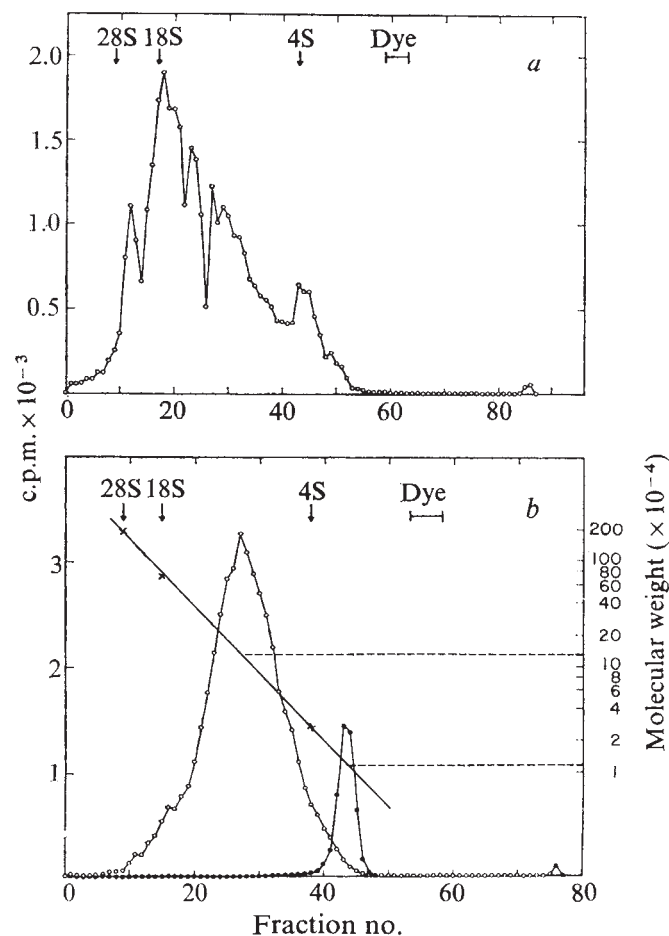


Fig. 2 Electrophoresis of the poly(A) sequence in 10% polyacrylamide gel in formamide. An aliquot of the poly(A) of experiment 7 (Table 1) was coelectrophoresed with HeLa ^3H -labelled RNA in a 12 cm, 10% polyacrylamide gel in formamide (98%) at 14 V cm⁻¹ for 4.5 h. After fractionating as in Fig. 1, ^{32}P was measured by Cerenkov radiation. Aliquots of fractions 8–20 were counted as in Fig. 1 to locate ^3H -labelled 5S and 4S RNA.

50% of total radioactivity was in mRNA, then poly(A)-containing RNA comprised less than 3% of mRNA.

Figure 2 shows an electrophoretogram of ^{32}P -poly(A) isolated after RNase T₁ treatment of the RNA. Poly(A) again migrated faster than the 4S and 5S RNA of HeLa cells. From its electrophoretic mobility in formamide gel, this ^{32}P -labelled poly(A) sequence was estimated to contain about 45 nucleotides.

The GMP content (Table 2) of the poly(A) sequence from RNase T₁ digested RNA shown in Fig. 2 indicates that poly(A) is near the 3' end of RNA, since there is far less than one GMP residue for a sequence of about 40 nucleotides. When hydrolysed with RNase T₁ and RNase A, the RNA yielded a poly(A) lacking UMP residues. The CMP and GMP residues found in this poly(A) could either be contaminants or nucleotides at the 3' end of the sequence. The first possibility is likely for CMP since the alkaline hydrolysis of poly(A) always produces about 1% of a component which comigrates with CMP during electrophoresis²¹.

Absence of these nucleotides from the 3' end of poly(A) was, however, shown more directly when a RNase T₁ and RNase A digest of RNA from cells labelled with ³H-adenosine released 6,000 c.p.m. in the poly(A) sequence of which 5,200 were recovered in AMP and 210 in adenosine. The average chain length of 25 calculated from these values was compatible with, although not identical to, the length of the ³²P-labelled poly(A) estimated by gel electrophoresis in Fig. 2. These data along with those of Table 2 show the poly(A) sequence of *E. coli* to be at the 3' end of the RNA as is the case for the mRNA of eukaryotic cells^{17,20,21}.

The detection in *E. coli* of poly(A) sequences covalently bound at the 3' ends of RNAs with characteristics of mRNA should eliminate speculation on roles for poly(A) sequences which define a function of eukaryotes not shared by prokaryotes, such as transport of mRNA from the nucleus. For the same reason, the presence of poly(A) sequences in mitochondria can no longer be used as evidence against the prokaryotic origin of mitochondria.

The concentration of poly(A) sequences in both pulse and fully labelled *E. coli* shows that, in contrast to animal cells and yeast, probably no more than 3% of mRNA molecules contain a poly(A) sequence long enough to bind to oligo(dT) cellulose. This difference is perhaps not surprising since the coupling of translation with transcription in prokaryotes may not allow the accumulation of many mRNAs with completed 3' termini. The low levels could also account for the failure of Perry *et al.*¹ to detect poly(A) sequences in *E. coli* mRNA, since they are close to the noise level reported for their experiments. In addition, the Millipore filter techniques they used would not have bound poly(A) sequences as short as those found here^{22,23}.

Although only a few mRNA species of *E. coli* have poly(A) at the 3' terminus, many mRNAs may have oligo(A) sequences at the 3' terminus which are too short to bind such RNA to oligo(dT) cellulose. Such adenylated 3'-termini have been found on the mRNAs synthesised *in vivo* after T₇ (ref. 24), as well as Φ80 phage²⁵ infection of *E. coli*. In both cases a variability in the yield of 3' adenylated mature mRNA species and the length of the oligo(A) sequence (usually 2 to 5 AMP residues) suggests that adenylation is post-transcriptional. Kramer *et al.*²⁴ suggested that oligo adenylation of the T₇ early mRNAs is a primitive counterpart of the poly adenylation found in eukaryotic mRNAs.

In considering a function for the polyadenylation of RNA molecules, it is interesting that the precursor of 5S RNA of *E. coli* may contain as many as seven uninterrupted AMP residues at the 3' end²⁶. Because 5S RNA has no known messenger function, a role for adenylation in RNA processing or maturation should perhaps be considered further, particularly since in spite of some recent elegant experiments, a role for poly(A) sequences in mRNA translation in eukaryotes has not been demonstrated²⁷.

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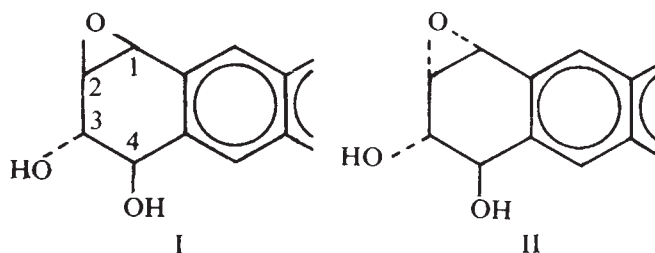
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Carbonium ion as ultimate carcinogen of polycyclic aromatic hydrocarbons

RECENT results have indicated that the proximate metabolites of benzo(a)pyrene¹ and benz(a)anthracene^{2,3}, two typical carcinogenic aromatic hydrocarbons, contain the 1,2-epoxy-3,4-dihydroxy-tetrahydronaphthalene moiety. This moiety arises from its parent hydrocarbon by formation of an arene oxide, enzymatic ring opening of this oxide to a diol, and then a second epoxidation. As the initially formed arene oxide is opened in a *trans* fashion⁴, only two geometrical isomers for the proximate metabolite are possible, and these are depicted as partial structures (I) and (II).



Here I discuss stereochemical features of these two isomers, and indicate that (I) can be predicted to have a much greater chemical reactivity with nucleophiles than (II). This is of interest because of the accepted theory^{5,6} that chemical carcinogens act by being metabolised to electrophiles which then react to form covalent linkages with nucleophilic groups present on DNA. Thus it becomes necessary to find metabolites that possess special features which lead to high reactivity in physiological conditions. I describe here that isomer (I) possesses a strategically placed OH group which is expected to assist the epoxide ring opening by neighbouring group participation and thus lead to substantially enhanced reactivity with nucleophiles. Furthermore, this OH group is expected to favour internal ion-pair formation and thus provide an electrophile of the appropriate character to react with DNA *in vivo*.

Both isomers (I) and (II) can exist in two conformations. In each case, however, only one conformation is favoured. The less favoured conformations involve eclipsing of bonds emanating from C₂ and C₃, the more favoured conformation of each isomer is shown in Fig. 1. This shows that the two hydroxyl groups are quasi-axially disposed in (I) and quasi-equatorial in (II). In particular the 4-OH group in isomer (I) is ideally situated for internal hydrogen bonding with the epoxide. This hydrogen bonding leads to a weakening of the C-O bonds on the epoxide and greatly facilitates nucleophilic attack at C₁ or C₂. The interatomic distance from the O of the

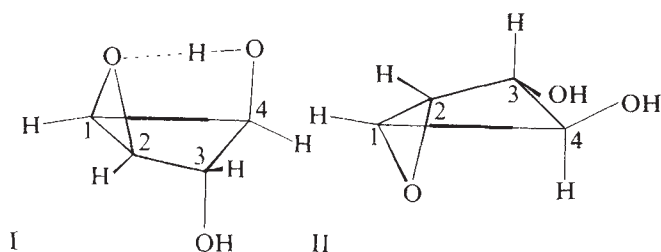
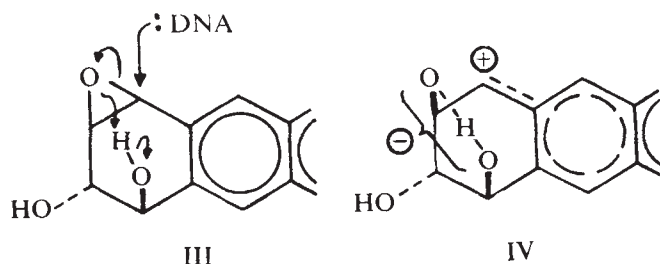


Fig. 1 Preferred conformations of partial structures (I) and (II), isomers of the 1,2-epoxy-3,4-dihydroxy-1,2,3,4-tetrahydronaphthalene moiety of the proximate metabolites of carcinogenic aromatic hydrocarbons. Conformations are viewed from a point equidistant from C_2 to C_3 and towards the molecule. Viewing point is in the plane of the aromatic ring but remote from it.

epoxide to that in the 4-OH group is 2.5 Å (Dreiding models). Generally epoxides require external acid catalysis for ring opening; however, with isomer (I) the catalysis will be intramolecular and thus the ring opening will occur rapidly under neutral conditions. In isomer (II), on the other hand, the OH groups are not appropriately oriented and such internally assisted epoxide ring opening is not possible, with the result that this isomer will be much less reactive.

Assistance by OH groups in the opening of epoxides by nucleophiles has been recognised in steroids⁷ and in the anti-leukaemic triptolide molecules⁸. In each of these examples, the OH group and the epoxide have the same structural and configurational relationships. These same relationships are also found between the 4-OH group and the 1,2-epoxide in (I). There is also conformational similarity as, in all three molecules, the key OH groups are in axial conformations. O—O interatomic distances of 2.7 Å (Dreiding models) and 3.1 Å (X-ray crystallographic work⁹), respectively, in the steroid and triptolide examples above, indicate that the distance of 2.5 Å in isomer (I) is short enough for strong interaction to take place.

The nucleophilic opening of the epoxide of isomer (I) is depicted formally in (III). Nucleophilic attack is possible at both C_1 and C_2 but attack at C_1 is shown because of the steric hindrance that the axial 3-OH group may cause, and because of the probable formation of the well stabilised internal ion pair (IV), in which the O anion is stabilised by the 4-OH group with the positive carbon centre stabilised by the aromatic nucleus. The positive carbon centre is in effect an aralkyl carbonium ion.



Internal ion pairs such as (IV) have already been shown to occur as the rate-limiting step in the spontaneous rearrangement of arene oxides^{10,11} in neutral conditions (which may be accompanied by the NIH shift¹²). In the case of (IV), however, the additional stability provided by participation of the neighbouring 4-OH group is a novel feature. Such additional stability is of course not possible in the analogous zwitterion from (II). The important consequence of the formation of the well-stabilised internal ion pair (IV) from isomer (I) is that this isomer will react with appreciable S_N1 character, that is, as a carbonium ion, rather than by an S_N2 mechanism. As a result it will react with a low sensitivity to the nucleophilic

strength of the incoming nucleophile and small but significant quantities of products from reaction with relatively poor nucleophiles, in the presence of stronger nucleophiles, will result. As DNA has low nucleophilic properties¹³, it therefore becomes a necessary feature of an ultimate metabolite that it can indeed react with appreciable carbonium-ion character so that it can alkylate DNA *in vivo*. The ion pair (IV) will react in this manner and thus has the appropriate electrophilic character to be considered as the ultimate metabolite of aromatic hydrocarbons. By contrast, the isomer (II) as a more typical arene oxide will react with much greater sensitivity to nucleophilic strength¹⁴, that is, in a predominantly S_N2 manner, and alkylate DNA to a negligible extent only. These general concepts have been elaborated previously¹⁵⁻¹⁸.

A more quantitative approach is possible if we arbitrarily assign Swain-Scott¹⁹ substrate constant values s to isomers (I) and (II). By analogy with those other carcinogens which act by way of carbonium ions (for example, isopropyl methane-sulphonate¹⁵, $s=0.29$; N-ethyl-N-nitrosourea¹⁷, $s=0.26$; and N-methyl-N-nitrosourea¹⁷, $s=0.42$), the s value of isomer (I) may be 0.3, whereas that of isomer (II) may be 0.9 (for example, glycidol¹⁹, $s=1.00$). Using the Swain-Scott equation¹⁹

$$\log(k_{\text{GSH}}/k_{\text{DNA}}) = s(n_{\text{GSH}} - n_{\text{DNA}})$$

relative rates of reaction with glutathione (a strong biological nucleophile and representative of other SH-containing compounds) and DNA can be compared. Values for the nucleophilicities n of SH groups and DNA are about 5.1 and 2.5, respectively¹⁵, and using these values it may be calculated that isomer (I) would react six times slower with DNA than with glutathione, whereas isomer (II) would react 220 times slower with DNA than with glutathione. Thus isomer (I) will alkylate a small but significant proportion of DNA, whereas isomer (II) will alkylate only a negligible proportion of DNA.

The sites of alkylation on DNA are multiple^{1,3}, and this also accords with the concept that the ultimate metabolite must have appreciable carbonium-ion character and consequently show poor discrimination for nucleophiles. A general value for the nucleophilicity of DNA was taken above, as it is not possible at this stage to consider any particular sites on DNA as critical. Aromatic hydrocarbons act by causing frame-shift alterations^{20,21}, in contrast to carbonium ions of smaller molecular size which cause base substitutions. With agents of this latter class, the very weakly nucleophilic¹⁶ O^6 group of guanine may be a critical alkylation site^{16,22}. Alkylation by a small group at this site interferes with DNA interstrand hydrogen bonding and base substitution occurs²³. Alkylation by large groups such as (IV) will clearly interfere more profoundly with the conformation of the double helix, by intercalation (see below) or by a 'base-displacement' mechanism²⁴, with frame-shift alterations resulting.

The extent of alkylation of DNA in the presence of other cellular constituents such as glutathione will depend not only on the first-order reaction rates described above, but also on the concentration of DNA and glutathione in the immediate vicinity of the ultimate metabolite. As the planar aromatic structure of the rest of the carcinogen molecule is expected to be predominantly intercalated^{20,21} with DNA, the concentration of DNA nucleophilic sites will be high compared to SH glutathione and protein sites. The ultimate metabolite thus has characteristics of an affinity label²⁵ for DNA, in that non-covalent binding encourages more specific covalent binding. The suggestion that many carcinogens and frame-shift mutagens may intercalate and then react covalently has been made^{20,21,26-28}.

The toxicities of bromobenzene and aromatic hydrocarbons are both mediated by the formation of an arene oxide metabolite, but whereas large amounts of bromobenzene are required to produce hepatotoxicity and this is seen only when glutathione is depleted²⁹, only very small amounts of aromatic hydrocarbons are required for a carcinogenic effect and no similar

threshold dose exists. This paradox can now be understood in the framework of the above discussion. Thus the 3,4-oxide of bromobenzene reacts in an S_N2 fashion, that is, selectively with strong nucleophiles such as glutathione. Only when body glutathione is depleted by reaction with an equivalent of bromobenzene oxide will this oxide react in appreciable quantity with the next most nucleophilic groups, which are on proteins. The toxicity arises as a result of this covalent reaction with proteins²⁹. DNA, a poor nucleophile, is presumably not attacked in the presence of proteins. On the other hand, I have described here how the arene oxide (I) from aromatic hydrocarbons can react as a carbonium ion with DNA, in the presence of glutathione and proteins, and thus no threshold need exist.

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Alternatives to the use of tabulated values of distributions in statistical programmes

SPECIFIC values from tables of statistical distributions that vary with degrees of freedom and probability (for example Student's t , χ^2 , correlation (r) or variance ratio (F)) are often included as input data, even to large detailed statistical packages^{1,2}. Alternatively, the complete tables are held in store. The former makes the program less flexible while the latter uses store inefficiency and is impractical for desk-top computers. A common alternative practice is to use approximate values; thus for example it has been suggested³ that for a sample number of thirty and more the values of t should be 2.0 when the probability (P) is 0.05. This value for $t(30-\infty)$ is within $\pm 2\%$ of the true value and was considered 'a close approximation [suitable] for most purposes'.

The alternative I propose is that simple equations should be formulated to calculate values for such statistical distributions to conserve store space and to make programs more adaptable. The equations should be relatively simple and within the present capabilities of small fixed-function desk-top computers.

As an example, the equation (1) is proposed as a simple approximate solution of Student's distribution of t . It was obtained by a trial and error fitting of the best equation-type

and curve to a graph of the distribution data. In this equation t_{Pn} is the value of t when the probability is P , for example 0.05, and the sample number is n , thus:

$$t_{Pn} = [\exp(k_P/(n-1))] + t_{P\infty} - 1.0 \quad (1)$$

in which the distribution of $t_{P\infty}$ is given by:

$$t_{P\infty} = -0.1093 (\log_{10}(1/P))^2 + 1.26 \log_{10}(1/P) + 0.4941 \quad (2)$$

The equation giving the values of k_P used to obtain optimum values of t in equation (1) over the range of probability 0.001-0.1 and using the values of $t_{P\infty}$ from equation (2) is given by:

$$k_P = [2.7497 \log_{10}(1.0/P)] - 1.1994 \quad (3)$$

This formula (1) provides values of t to within $\pm 5\%$ of the true value⁴ for a sample number of between three and infinity and within the range of probabilities $P = 0.1-0.005$; values of t for a sample number of thirty and over are within $\pm 1\%$ of the true t . Values of t over the wider probability range of $P = 0.2-0.001$ were within $\pm 8\%$ of the true t for sample numbers of four to infinity; sample numbers over 30 were within $\pm 3\%$ of the true t .

I realise that when the confidence or fiducial limits thus obtained do not show a clear decision the true tables must be consulted or the equation suggested must be refined; but in most cases these small errors are of little consequence.

The correlation coefficient, r , can also be calculated knowing t for n pairs of samples at the probability P using the equation below (modified from Fisher and Yates⁵):

$$r^2 = t^2 / [(n-2) + t^2] \quad (4)$$

Answers to statistical problems are usually presented in terms of a few standard values of probability, for example the t test confidence intervals. An iterative process could be used with suitable formulae to express the differences between sets of samples in the form of an exact probability, for example $P = 0.06$. With the present formula, results are only reliable within the range of probability 0.1 to 0.005. The use of an approximate formula to calculate the critical values of F could then result in a matrix of probabilities for a comparison of several sets of data. With desk-top computers, the latter step will depend on the demand to make statistical distributions available as fixed functions.

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Erratum

In the article "Binding of flexible ligands to macromolecules" by A. S. V. Burgen, G. C. K. Roberts and J. Feeney (*Nature*, 253, 753; 1975), the equation at the foot of the first column on page 754 should read.

$$\Delta F_1 = -RT \ln K_1 = \Delta H_1 - T(\Delta S_{ir} + \Delta S_{rot} + \Delta S_{conf.})$$

and not as printed.

matters arising

Origin of cellular senescence

I HOPE to clarify a point regarding the recent letter of Dykhuizen¹. He speculated that cellular senescence is the result of a selection process imposed by an organism's need to ensure its survival by limiting the size of atherosclerotic plaques which, he believed, originated from relocated fibroblasts. This is an interesting new view of the limit on fibroblast doubling potential described by Hayflick² and others, and the predictions he derives from the hypothesis can be tested experimentally. These include the ultimate cessation of plaque growth when cells at the edge senesce, higher division potential of cells isolated from the centres of plaques, and a correlation between final plaque size and the age of the organism at the plaque's initiation. The fulfilment of these predictions will shed some light on the (probably fibroblastic) origin of plaques but is not critically dependent on an evolved origin for cellular senescence (or doubling potential limit) in the manner Dykhuizen suggests.

The expected observations could be explained, without resort to an evolutionary origin, simply on the basis of known facts. Specifically, cellular senescence manifests itself through a limit on the doubling capacity of fibroblasts; such cells are subject to "contact inhibition" of division³, and the doubling potential of fibroblasts is related to the age of the donor³. Whatever the ultimate cause of cellular senescence or its origin, the behaviour, given these facts, will be the same if plaques do arise from fibroblasts.

The predictions thus do not differentiate between Dykhuizen's explanation of an evolved origin for cellular senescence and a number of other processes which have been postulated as the primary cause of ageing such as error accumulation, a specific genetic programme or the exhaustion of a developmental programme.

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² Hayflick, L., *Expl Cell Res.*, 37, 614-636 (1965).

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DYKHUIZEN REPLIES—The lack of an understandable selective advantage for cellular senescence has been a major criticism of a hypothesis for its evolutionary origin¹. The main point of my paper² was to provide a reasonable

evolutionary explanation. To do this, I presented a novel theory of the formation of atherosclerotic plaques. The predictions were meant to test this theory which, if correct, implies that the size of plaques is limited by cell senescence, providing a possible selective advantage to the organism.

Given the assumption that a specific genetic programme could only arise through evolution, the evolutionary origin of cell senescence could be demonstrated by finding a gene, which, when rendered non-functional, allows cells which would normally senesce to become immortal. The error and evolutionary hypotheses could possibly be distinguished by determining the percentage of daughter cells of recently divided cells which go on to divide in a senescent culture and in an immortal culture derived from otherwise similar cells. Orgel¹ suggested that if his error theory holds, then both percentages should be near 50%—slightly less in the senescent and slightly more in the immortal culture. If the hypothesis of evolutionary origin is correct, these percentages should be quite different, say, 20% and 90% respectively.

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¹ Orgel, L. E., *Nature*, 243, 441-445 (1973).

² Dykhuizen, D., *Nature*, 251, 616-618 (1974).

Further note on electron diffraction symmetries

IN connection with diffraction symmetries it has been suggested¹ that there are two conditions for a centrosymmetric pattern though there is some contention that the first of these conditions is incomplete. As the chief protagonists in this debate we wish to clarify the situation with respect to zero-beam symmetry and reciprocity.

We shall consider first those symmetries observable in convergent-beam zone-axis patterns, that is, restricted sections of reciprocal space distributions disposed on a plane with the geometry of the diffraction pattern. Under these restrictions, a rotation diad along the zone axis completely determines a centrosymmetric pattern for all beams, that is, it is a sufficient condition for the whole pattern and does not require any consideration of reciprocity. (Note that to preserve the diad symmetry consideration must be restricted to either parallel-sided foils lying perpendicular to the incident beam, or else to crystals with boundaries which conform to the diad symmetry.)

This covers the example given by

Steeds² and requires a correction to the table presented by Goodman³. To the last column of entry (2) in the table should be added the words "unless there is a rotation diad parallel to the zone, in which case the pattern will be centrosymmetric". Consideration of reciprocity allows general conditions to be found for zero-beam symmetry which do not apply to the remainder of the pattern as a whole but also give the internal symmetry of each dark-field intensity distribution. Thus, a central mirror or glide plane (condition 1 of Goodman¹) is a sufficient condition for centrosymmetry in the zero-beam pattern, but not in the remainder of the pattern. For other symmetries, a rotation diad or twofold screw axis, midway between the surfaces and parallel to them, ensures a mirror line in the zero-beam pattern (though not in the remainder of the pattern), perpendicular to the diad or screw axis (though this does not ensure a centre of symmetry in the zero-beam pattern). Higher symmetries than a mirror line or a centre can be built up by combination. Derivation of these rules from the invariance properties of the crystal does not require an explicit solution for the wave function, and can be applied to a crystal of any structure or surface geometry.

From such rules one can proceed to derive a description of a zone-axis pattern for a particular space group. But considerations of electron diffraction always concern crystals that are finite in at least one direction, and the strong interaction of electrons with the crystal potential makes it impossible to ignore the boundary conditions, so the symmetry of the total crystal must, therefore, be considered. The influence of tilted surfaces may be avoided to any accuracy by choosing crystal foils sufficiently parallel-sided and horizontal (say in the range of 0-20°). But the influence of crystal termination in a flat horizontal specimen will mean that the crystal may not have the three-dimensional symmetry of the space group, and in such cases the crystal boundaries used in a symmetry derivation need to be stated.

Experimental limitations need to be considered. Convergent-beam diffraction, and diffraction-contoured micrographs, are practical methods of symmetry analysis with individual limitations. The pattern symmetries can be expressed by two-dimensional point-group symbols, whether investigating two-dimensional projection symmetries or three-dimen-

sional symmetries of the crystal. In the first approximation, the two-dimensional symmetry of the projection at the zone determines the diffraction symmetry and the symmetry of the pattern only has the symmetry of the projection. When measurements are sufficiently accurate, or cover a sufficient angular range, the symmetries must be derived from the three-dimensional space group in the way indicated.

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Binding of 25-hydroxy vitamin D₂ to plasma protein in New World monkeys

BELSEY *et al.*¹ have shown that the lower potency of ergocalciferol (D₂) compared with cholecalciferol (D₃) in chicks is reflected in a weaker binding of D₂ metabolites to the plasma transport proteins. Further investigations in other species known to be resistant to D₂ were recommended¹ to establish whether similar correlations exist between biological activity and binding to transport proteins. Since New World primates (Cebidae) utilise D₂ less efficiently than D₃ (refs 2-4) we examined the transport proteins in four species of New World monkeys to test whether they also discriminate against D₂ metabolites.

A competitive protein binding assay involving the displacement of ³H-25-OH-D₃ from the specific plasma transport proteins by 25-OH-D₂ and 25-OH-D₃ was used to test the relative binding of these two metabolites of D₂ and D₃.

In the assay system with plasma from the monkey *Cebus albifrons*, increasing amounts of 25-OH-D₂ and 25-OH-D₃ produced similar reductions of the bound ³H-25-OH-D₃ (Fig. 1) indicating equivalent binding of the two steroids by the *C. albifrons* transport protein. A similar result was obtained for the three other primates tested—*C. capucinus*, *Aotus trivirgatus*, and *Callithrix jacchus*. Old World monkeys (Cercopithecidae) utilise D₃ and D₂ with equal efficiency³ and in the three Old World primate species we studied (*Erythrocebus patas*, *Macaca mulatta*, and *Papio anubis*) the transport proteins exhibited equal affinity for 25-OH-D₂ and 25-OH-D₃.

There are differences in the type of protein used by the species examined in this study for vitamin D transport. *C. albifrons* and *C. capucinus* use albumin⁶ whereas the two other New World and

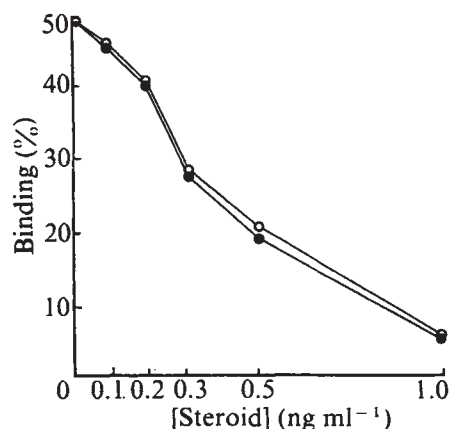


Fig. 1 Competitive displacement of ³H-25-OH vitamin D₃ from *C. albifrons* plasma with increasing amounts of non-labelled vitamin D analogues. Each point is the mean of two determinations. ○, 25-OH-D₂; ●, 25-OH-D₃.

the three Old World primates use an α -globulin fraction⁶. The seven primates studied only have a single transport protein⁶ whereas the chick has two proteins which exhibit β -globulin mobility on gel electrophoresis^{6,7}. Although two types of protein are used for vitamin D transport in these primates their binding properties for 25-OH-D₂ and 25-OH-D₃ are similar.

The results obtained for the steroid binding properties of the New World monkey transport proteins are in contrast to those of the chick proteins which do discriminate against D₂ metabolites¹, an observation we were able to confirm. From this, it would seem that the resistance of New World primates to vitamin D₂ is a result of other differences. These may involve inefficient binding of D₂ and 25-OH-D₂ by intracellular vitamin D binding proteins in liver or kidney respectively or discrimination against the active kidney metabolite 1,25-(OH)₂-D₃ by the binding proteins present in target organs such as muscle, gut and bone.

The variations between the binding properties of New World primate and chick transport proteins for 25-OH-D₂ illustrate the difficulty of postulating a general theory of D₂ resistance. These differences confirm that the process of discrimination against D₂ in species resistant to this vitamin cannot be explained uniformly by the properties of the plasma protein as evidenced by the chick. It seems, therefore, that a wider survey of vertebrates known to be resistant to D₂ is required to correlate the random observations made in individual species.

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Ultraviolet viewing with battery-operated television camera

LAVIGNE and Oritsland¹ have shown that ultraviolet photography can be used usefully for the detection of white animals on snow. Polar bears, for example, ordinarily match their background and may be difficult to spot, certainly from a distance, but when photographed in the near ultraviolet band of the electromagnetic spectrum (300-400 nm) they appear as black bodies, sharply contrasted against the snow. The technique is said to have application for the remote sensing of polar bears in the field. We suggest that ultraviolet detection can also be carried out conveniently with a television camera. Such cameras are intrinsically sensitive to the near ultraviolet band, and to be used for ultraviolet viewing need only be outfitted with an ultraviolet-transmitting lens and filter. We have used the technique for viewing ultraviolet contrast patterns on flowers and butterflies², but it should also lend itself to detection on snow of animals which absorb in the ultraviolet. Though conventional, portable video systems, suitable for use in the field, include both a camera and a recorder, the recorder can be disconnected easily, and the camera can be converted to a self-contained viewer if it is fitted with a battery pack attached to the housing. The disconnection of the recorder decreases the vertical resolution of the camera's monitor (because of the loss of scan synchronisation pulses from the recorder), but the quality of the image is adequate for most purposes (full resolution can be restored by the installation of an oscillator and appropriate divider circuits on the camera). Such cameras provide compact, manageably light, and relatively inexpensive, ultraviolet image transducers.

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reviews

THERE comes a stage in the development of any powerful new experimental technique, microscopical or otherwise, when its value for research is so well established that an authoritative survey is essential to its full exploitation in science and technology generally, beyond the circle of enthusiastic pioneers. Transmission electron microscopy has long reached that position, and the need is being satisfied by a number of monographs and treatises, including Professor Hayat's *Basic Electron Microscopy Techniques* (a conveniently short volume) and his *Principles and Techniques of Electron Microscopy: Biological Applications*, published by the same house. Whether his *Electron Microscopy of Enzymes* (two volumes, so far) is equally timely I am not qualified to judge. But I am in little doubt that a similar treatment of the biological applications of scanning electron microscopy is at best premature and at worst likely to be misleading. There simply has not been enough work done in the subject to serve as basis for it. Only a limited number of biologists throughout the world are seriously applying themselves to the problems of specimen preparation and observation, as distinct from the exploratory use of the instrument.

However useful it may be to newcomers in the field, the title borne by Hayat's new series covering scanning electron microscopy is a misnomer. In effect, it is a journal of biological scanning electron microscopy, largely devoted to articles describing the work of the individual contributors. True, there is an article descriptive of the instrument itself, compressed into 42 pages and failing to mention the two major treatments by Hearle *et al.* (1972) and Oatley (1972). There are also excellent descriptions of the critical point drying technique (60 pp.) and of the preparation of stereomicrographs (14 pp.), both of which are deserving of a wider readership. Nearly all the remaining 19 articles are short accounts (averaging 17 pp.) of special applications of the instrument in the study of spores, leaf surfaces, plant cell walls, wood, intracellular structures, marine teleosts, ciliated epithelia, lung, bone, and so on. To do him justice, the editor is quite frank in his preface to the second volume: "This treatise [*sic*] departs from the tradition that a book on methodology presents only the contemporary consensus of knowledge. It is written by scholars [who have] anticipated the potential usefulness of a new method."

Microscopy

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Principles and Techniques of Scanning Electron Microscopy: Biological Applications. Edited by M. A. Hayat. Vol. 1: Pp. 273; £11.95. Vol. 2: Pp. xii+171; £10.60. (Van Nostrand Reinhold: New York and London, October 1974.)

Principles and Techniques of Electron Microscopy: Biological Applications. Edited by M. A. Hayat. Vol. 3: Pp. xii+321; £11.35. Vol. 4: Pp. xi+216; £10.60. (Van Nostrand Reinhold: New York and London, November 1974.)

If I had to advise a library on its buying policy, my opinion would be that Volume 1 may be worth its price as an introduction to the subject if *The Use of the Scanning Electron Microscope* by Hearle *et al.* is not already on the shelves, but that Volume 2 should only be bought if urgently requested by local biologists. They would do better, however, to consult the original papers in their field of interest, most, if not all, of which they will find listed in the *Bibliography on Biomedical Applications of Scanning Electron Microscopy* compiled by Boyde *et al.* and published in *Scanning Electron Microscopy* 1973, the proceedings of one of the annual meetings on the subject at the Illinois Institute of Technology.

Principles and Techniques of Electron Microscopy: Biological Applications (Volumes 3 and 4) can be viewed more favourably. This series, now planned to extend to eight volumes, sets out to cover the whole range of knowledge involved, including the operation of the microscope itself in its various modes.

The current volumes contain seven and eight articles, respectively. They vary greatly in nature and style, from a broad survey of a topic to a monograph on an author's special field of work. Understandably, the latter form is usually the more readable and useable, and includes articles on high resolution, dark-field microscopy, in-focus phase contrast and stereological techniques in Volume 3, and in Volume 4 on optical shadowing, relative mass determination in dark-field, microscopy of single cultured cells, denaturation mapping of DNA and the examination of polysomes from cardiac muscle. At the other extreme lie collections of recipes for specimen preparation: selective staining of molecules, sub-

cellular fractionation in the ultracentrifuge, and critical point-drying (surely the hyphen is misplaced?) in Volume 3; and ultramicroincineration in Volume 4. The latter volume also contains a comprehensive if uncritical account of preparatory [*sic*] methods for electron probe analysis by X-ray spectrometry. Two articles fall outside these categories: a general introduction to the electron microscope and its operation in Volume 3 and an account of methods for counting virus particles in Volume 4.

Granted that the subject is now so wide ranging as not to be encompassed in a treatise by a single author, the best method of covering it by multi-authorship has to be solved. The treatment planned by the editor of this series falls somewhere between two others now becoming available: the encyclopaedic *Methodensammlung der Elektronenmikroskopie* (Wissenschaftliche, Stuttgart) and *Practical Methods in Electron Microscopy* (North-Holland, Amsterdam). The former breaks down electron microscopy into a great number of sub-topics, treated at a high level of expertise in relatively short compass, whereas each volume of the latter comprises two or three long articles in which a rather broader subject is dealt with in greater depth. Dr Hayat's series covers much the same subject matter, but unevenly and rather randomly. Apart from the first two volumes, which described basic preparative techniques, the two under review (and the remaining four as listed) contain an unrelated assortment of contributions. Some of these are by acknowledged leaders in a subject, others by recipe collectors. The level of approach varies from elementary to advanced; the proof reading varies from excellent to execrable (even the editor's name is misspelt in one place); and the current-awareness varies from yesterday to the day before. In such a rapidly moving subject it is regrettable to find few references in Volume 3 later than 1970 or in Volume 4, later than 1971, apart from cross-references within the series.

That said, these volumes have the merit of purveying in compact form a great deal of information, some of value to present practitioners and most of help to the beginner. He, she or they are likely to decide that one or the other of them is sufficiently valuable to be bought, even at these rather high prices. The whole series ought to be available in any biological laboratory making regular use of the electron microscope. □

Welcome addition to multivariate analysis

Discrete Multivariate Analysis: Theory and Practice. By Yvonne M. M. Bishop, Stephen E. Fienberg and Paul W. Holland. Pp. x+557. (MIT Press: Cambridge, Massachusetts, and London, March 1975.) \$27.50.

DISCRETE data, in which each variable takes one of a limited number of possible forms, arise frequently in many fields. Multivariate analysis of such data is required when the joint distribution of several variables is to be studied. The present book gives an account of statistical methods for these problems. It concentrates largely, although not entirely, on an approach in which the log frequencies in cells of a multi-dimensional contingency table are represented in some simple form, the so-called log linear model. To an appreciable extent the book is based on the authors' earlier important contributions to this topic.

The discussion is lucid and very leisurely, excellently illustrated with applications drawn from a wide variety of fields. A good part of the book can be understood without very specialised statistical knowledge. It is a most welcome contribution to an interesting and lively subject.

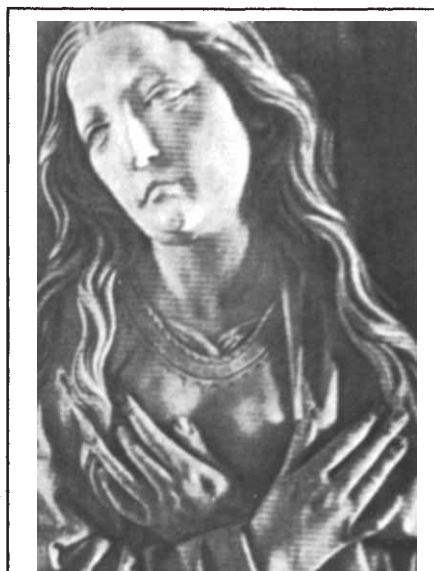
After an introductory chapter, structural models for multidimensional discrete data are described, and then maximum likelihood methods of estimation are developed; this is followed by a consideration of the methods used for testing degree of fit. Methods for incomplete data (missing cells) are discussed at length. There is an interesting chapter on estimating the size of a closed population, in which the usual methods for the analysis of a multiple capture-recapture census are modified to take account of possible dependencies between the samples on successive occasions. Further chapters include discussions on Markov models for assessing change and on the special problems of 'square' tables. The book ends with two chapters on approaches other than that of the log linear model and chapters on pseudo-Bayes estimates (that is, on smoothing tables), on sampling models and on mathematical aspects.

The scope of the book is rather less than might have been expected from its title. For instance, there is no discussion of latent structure analysis or of the difficult problems of analysing multivariate discrete data in connection with numerical taxonomy.

I have one general criticism, which, though to some extent a matter of taste, seems to me important. The

authors barely mention the distinction between response variables (dependent variables) and explanatory variables (independent variables). Yet in quite a number of their examples the point at issue seems to concern how variation in one of the variables is explained by the other variables, which is a univariate and not a multivariate problem. Even if the detailed analysis is little affected, incisive explanation of conclusions is much easier for univariate problems. At a more technical level the authors have ignored the fact that in some—but not, of course, all—of their problems conditioning on ancillary statistics is possible.

D. R. Cox



The Virgin Mary, hands crossed in the gesture of humble submission. A wood carving from the Marienalter of Herrgottskirche in Creglingen, Germany. Photograph taken from *Silent Language*, by Macdonald Critchley. Pp. vi+231. (Butterworth: London, April 1975.) £9.50.

Fundamentals

Fundamentals of Mathematics. Vol. 1: *Foundations of Mathematics/The Real Number System and Algebra.* Pp. x+549. Vol. 2: *Geometry.* Pp. xi+685. Vol. 3: *Analysis.* Pp. xiii+541. Edited by H. Behnke, F. Bachmann, K. Fladt and W. Suss. (MIT Press: Cambridge, Massachusetts, and London, 1974.) \$15.95 each volume; \$40.00 the set.

THIS work stands alone; it is a unique survey of the whole field of pure mathematics, with emphasis on the basic ideas and their interrelationships. For its origin, we must go back over 20 years. In 1954 the International Commission for Mathematical Instruction, at a meeting in Paris, chose the scientific foundations of instruction in mathematics as one topic for the 1958 International Congress

in Edinburgh. As a result, the German section of the Commission began Volume I of this work. During its subsequent development authors from various European countries contributed, and it became clear that the material would be important not only for teachers and lecturers but also for research mathematicians. The original publication was in German, and the volumes now reviewed consist of a translation based on the second German edition of 1962; it incorporates various improvements and exercises designed to bring the account up to date.

Each chapter has been written by two authors, one a university research mathematician, the other "a teacher of long experience in the educational system". As a result, over 80 authors have contributed.

Volume I consists of a part on the foundations of mathematics and a part on arithmetic and algebra. Volume II deals with geometry, and consists of 16 chapters covering both the foundations and the analytic treatment of this aspect of mathematics. Worthy of special mention are the chapters on geometrical constructions and on polygons and polyhedra. Volume III is concerned with analysis and includes 14 chapters.

No mathematical specialist can consult the work without becoming acutely aware of the tremendous breadth of mathematical thought and discovery. The student, contemplating research in some area of the subject, would do well to read, in these volumes, of the relationship between that particular area and the subject at large; for it is a distinctive feature of the work that it emphasises the connections between different branches of mathematics.

Many people, including a lot of mathematicians, find it difficult to see perspective in mathematics. To such, the last chapter, on the changing structure of modern mathematics, is to be commended. Here is a very clear account, carefully traced, of what has been happening in recent decades. It is all summed up in the expression "axiomatic way of thinking". There is reference, of course, to the Bourbaki movement in France, and to the development of the axiomatic method in geometry. Then comes the change in algebra, namely the breakthrough of axiomatic algebra (covered particularly well by E. Artin and Emmy Noether). Finally, the great achievements of the axiomatic method in analysis are traced, although these considerations are tinged with an element of doubt as to whether the axiomatic change in analysis will ever be as complete as that in algebra.

No serious mathematician can afford to neglect these volumes. To read them is an experience; and, at the end, the reader will be more aware of the abiding serenity of his subject.

L. S. Goddard

The Heritage of Copernicus: Theories "Pleasing to the Mind". (The Copernican Volume of the National Academy of Sciences.) Edited by Jerzy Neyman. Pp. 542. (MIT Press: Cambridge, Massachusetts, and London, 1974.) n.p.

This book is not about Copernicus, nor about the postulated revolution to which he has given his name. Rather, it is a collection of 24 essays, by various hands, dealing with various other postulated 'Copernican' or 'Quasi-Copernican' 'revolutions' in astronomy, cosmology, biology, chemistry, physics, mathematics, probability theory and technology. The book is intended for the general reader, who will need to have a rather large technical vocabulary, or access to a rather large and up-to-date dictionary. None of the contributors seems to be a professional historian of science.

Many of the 'revolutions' described are merely good pieces of traditional science—such as Shapley's work on our Galaxy—and almost all the references to Copernicus' own work show a degree of incomprehension that makes the book highly ambiguous as a tribute.

The most interesting essays are those which pay least attention to Copernicus or to 'revolutions': one by Geoffrey and Margaret Burbidge on some of the problems facing modern cosmological theories, and one by Stanley L. Miller on "The First Laboratory Synthesis of Organic Compounds under Primitive Earth Conditions". **J. V. Field**

The Laue Method. By Jose Luis Amoros, Martin J. Buerger and Marisa Canut de Amoros. Pp. xi+375. (Academic: New York and London, March 1975.) \$37.00; £17.75.

THE Laue technique, although the oldest used by X-ray diffractionists, was early superseded in many areas of structure analysis by methods based on monochromatic radiation and moving crystals. But it has remained a useful technique in metallurgy and crystal physics, especially, though no thorough presentation of its potentialities has appeared for about 40 years. This book aims to fill the gap.

Chapter 1 is historical, chapters 2–8 are written especially for the numerous users who have had no formal training in crystallography, and chapters 9–13 are aimed at the specialist. Structure analysts who normally only take Laue photographs by accident will find this book well worth reading, partly for the clarity of its presentation, but also because it presents some fresh and some neglected ideas. The novel suggestion for the use of the method in crystallochemical analysis seem to me to be rather too opti-

mistic, but the discussion of the idea that cylindrical Laue photographs can be used to determine the symmetry and orientation of an arbitrarily set crystal will well repay study. So too will the sections which discuss the methods used in the study of the diffuse scattering which results from thermal motion, disorder or crystal imperfections.

The last chapter purports to present a new method of interpreting the photographs; I can remember it being used, however, for teaching the topic at a summer school in 1947 and can attest to its utility. **D. Rogers**

Books brief

Design Theory of Fluidic Components. By Joseph M. Kirshner and Silas Katz. Pp. xi+479. (Academic: New York and London, January 1975.) \$45.00; £21.60.

At present the list of books concerned with the relatively new technology of fluidics is quite short. Kirshner and Katz have made a valuable addition to the list. The initial chapters about the properties of fluid filled transmission lines and jet flows are particularly good and the bibliographies given after each chapter are extensive and well up to date. The authors have rightly restricted their attention to devices with non-moving parts and devote a complete chapter to each main type and their performance characteristics (these include the impact modulator, the vortex triode, the beam deflection amplifier, the bistable switch and the transition NOR). The appendices contain data on air filled transmission lines and also computer programs for calculating the response of such lines to various inputs. These will be very useful to system designers. Likewise, newcomers and students will find the list of problems following most chapters helpful, though for some reason the answers are not given. **Terry Hughes**

Mycotoxins. Edited by I. F. H. Purchase. Pp. xiii+443. (Elsevier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl.115; \$44.25.

THE discovery of aflatoxin 15 years ago attracted the interest of many scientists to the field of the mycotoxins. It was indeed fortunate as previously very little had been known of the chemistry and mode of action of the mycotoxins in spite of numerous ancient descriptions

of the massive poisonings of people and domestic animals by these fungal metabolites.

This book, addressed primarily to investigators and students, contains ample information on different aspects of studies on mycotoxins. Contributors have not followed precisely unified criteria in their presentations and some chapters are treated from widely different points of view. Furthermore, there are four chapters concerned with trichothecene mycotoxins, which results in some overlapping in the information on these compounds.

The authors have been chosen well. Most of them are leading authorities in the subjects which they write about, and the coverage is very good although there are few data dating from later than the end of 1972.

The presentation and typography of the book are excellent.

This volume should be useful not only to mycologists and scientists interested in mycotoxins but also to pathologists and experts in bromatology and toxicology. The book might be very encouraging to investigators interested in the mode of action of mycotoxins, since little work has been done on this aspect of these compounds. **David Vazquez**

Handbook of Genetics. Vol. 1: *Bacteria, Bacteriophages and Fungi*. Edited by Robert G. King. Pp. xvi+676. (Plenum: New York and London, 1974.) \$44.50.

THIS book, the first in a series, aims to review organisms which have been extensively used by geneticists. It is an excellent collection of review articles interspersed with techniques, gene symbols and chromosome maps for selected bacteria, bacteriophages and fungi. The introductory article on a classification and evolutionary scheme for all organisms, although interesting and relevant to the series as a whole, seems out of place as it makes no reference to techniques such as %G+C and numerical taxonomy. Most of the articles are of a high standard and contain a wealth of technical and background material. The total omission, however, of any reference to the extensive genetical work on *Coprinus lagopus* in an article entitled 'Coprinus' is difficult to understand but this is an isolated case and the rest of the material is well balanced. The usefulness of the indices could have been improved by giving textual pages rather than bibliographical pages in the author index and by greater cross referencing in the subject index.

In spite of these relatively minor criticisms and the high price, this book should become a basic reference work for research workers and teachers in microbial genetics. **B. W. Bainbridge**

obituary

Robert Alexander Houstoun, a leader in the optics field, died on May 4 at the age of 92.

A student of Glasgow, he pursued his studies at Cambridge and Göttingen, where he obtained his PhD. Joining the staff of Glasgow's natural philosophy department in 1906 he continued his research in optics till he retired in 1948 and for some years after as an Honorary Research Fellow. His research interests were varied but he is best recalled for his work in colour vision and the measurement of the velocity of light in air and water by the piezo-quartz shutter method, which he developed. He also found time to write a considerable number of successful books. His *Treatise on Light*, first pub-

lished in 1915, was for a long time the standard work in its field, and a considerable number of published papers flowed from his pen from 1904 to the end of the 1960s.

Eric H. Lenneberg, professor of psychology and neurobiology at Cornell University, has died at the age of 53.

At the time of his death, Dr Lenneberg was working on a clinical research project in neuropsychology at the Westchester Division of New York-Cornell Medical Center, after having joined Cornell in 1968. His major scientific achievement was considered to be the proposal, first advanced by him in the 1950s, that the human capacity for

language could be explained only on the basis of the biological properties of the brain and vocal tract. He had summarised his experiments and views in his book *Biological Foundations of Language*, published in 1967. Dr Lenneberg was born in Germany and after spending much of his youth and childhood in Brazil, went to the US in 1945, eventually obtaining degrees from Columbia and Harvard. He joined the staff of Harvard, and later carried out basic research on language development in defective children at the Children's Hospital Medical Center in Boston while on the staff of the Massachusetts Institute of Technology. From 1967-68, he was professor of psychology at the University of Michigan.

announcements

Awards

The Royal Institute of Chemistry has made the following awards: **Meldola Medal and Prize** for 1974 to **P. J. Derrick** for contributions to the development of field ionisation kinetics and mass spectrometry; **Beilby Medal and Prize** for 1975 to **P. R. Swann** for work in electron microscopy of metals alloys and phase transformations.

Reports and publications

Great Britain

The Annual Report of the Science Policy Research Unit at the University of Sussex for the year 1974. Pp. 60. (Falmer, Brighton: SPRU, University of Sussex, 1975.) [15]

Advisory Committee on Oil Pollution of the Sea. Annual Report for 1974. Pp. 28. (London: Advisory Committee on Oil Pollution of the Sea, 39 Victoria Street, 1975.) [15]

Report by the Hydrographer of the Navy, Rear-Admiral G. P. D. Hall, for the year 1974. Pp. 40. (London: Ministry of Defence, 1975.) [55]

Grassland Research Institute. Technical Report No. 16 March 1975. The Construction of Instruments for Measuring and Manipulating the Plant Environment. By J. E. Sheehy and A. M. Tearle. Pp. 36. (Hurlay, Maidenhead, Berks: The Grassland Research Institute, 1975.) £1.50. [55]

Royal Greenwich Observatory. Greenwich Time Report, Time and Latitude service 1974 January-June. Pp. 36. (Herstmonceux: Royal Greenwich Observatory, 1975.) [85]

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Institution of Gas Engineers. Communications. No. 955: Fifth Report of the Education and Training Committee, 1974/75. Pp. 26. £1. No. 956: Presidential Address by A. G. Pratt. Pp. 35. £1. No. 957: Technical Aspects of Natural Gas in Victoria. By J. M. Shaw. Pp. 24. £1. No. 958: Developments in Industrial Service in the West Midlands. By K. Manuel and J. W. Price. Pp. 26. £1. No. 959: Segas—a Suitable Case for Modelling. By H. W. D. Hughes and R. J. Martin. Pp. 26. No. 960: Gas from Coal for Synthesis of Hydrocarbons. By J. C. Hoogendoorn. Pp. 13. £1. No. 961:

The Development of a Computer Assisted Customer Service System. By H. N. Coates and A. L. Hardy. Pp. 21. £1. No. 962: The North Sea and the British Energy Scene. By J. G. Liverman. Pp. 14. £1. No. 963: Money—at What Price? By J. H. Smith. Pp. 32. £1. No. 964: The SBGI and the Gas Appliance Industry—Its Role in Britain and Europe. By A. J. Adam. Pp. 17. £1. No. 965: Engineering for Expansion. By G. F. E. Roberts. Pp. 28. £1. (London: Institution of Gas Engineers, 1975.) [125]

Meteorites: a Concise Account. By A. A. Moss. Pp. v + 26. (London: British Museum (Natural History), 1975.) 35p. [125]

Structural Analysis and Structural Failure. By Professor E. H. Brown. (Inaugural Lecture, 13 November 1973.) Pp. 183-198. (London: Imperial College of Science and Technology, University of London, 1975.) £1.50. [125]

Proceedings of the Royal Society of London. B: Biological Sciences, Vol. 189, No. 1095: A Discussion on the Recognition of Alien Life. Organized by N. W. Pirie, FRS. Pp. 137-274. (London: Royal Society, 1975.) UK £3.20; Overseas £3.30. [125]

Some Results of Research in Chemical Engineering and Technology Supported by the Science Research Council. Pp. 15. (London: Science Research Council, 1975.) [145]

Multiple Sclerosis. Pp. 40. (London: Office of Health Economics, 162 Regent Street, 1975.) 25p. [155]

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Competition, Risk and Profit in the Pharmaceutical Industry. (An Economic Review commissioned from Runnymede Research Limited and based on Studies by George and Priscilla Polanyi.) Pp. 53. (London: The Association of the British Pharmaceutical Industry, 162 Regent Street, 1975.) [165]

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Other countries

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